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(54) Title: NEW HBV SEQUENCES

(57) Abstract: The complete nucleic acid sequence of a new human hepatitis B virus genotype, provisionally named genotype G, is reported. This genotype was found with a high prevalence in patients chronically infected with HBV and residing in Europe and the USA. The present invention further relates to polypeptides encoded by said nucleic acid sequence and to antibodies recognizing said polypeptides. The present invention also relates to the use of said nucleic acid, polypeptides and antibodies in HBV diagnosis, prophylaxis and therapy.

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NEW HBV SEQUENCES

FIELD OF THE INVENTION

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The present invention relates to the field of Hepatitis B virus (HBV) diagnostics and therapeutics. The present invention provides sequences of a new HBV genotype G and a new mechanism for HBeAg modulation for use in diagnosis, prevention and treatment of HBV infections.

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BACKGROUND OF THE INVENTION

Human Hepatitis B virus (HBV), which is the prototype member of the family *Hepadnaviridae*, is a circular, partially double-stranded DNA virus of approximately 3200 nucleotides (Magnius and Norder, 1995). This highly compact genome contains the four major open reading frames (ORFs) encoding the envelope (preS1, preS2 and surface antigen HBsAg), core (preCore precursor protein, HBeAg and HBcAg), polymerase (HBpol) and X (HBX) proteins, respectively.

By using subtype-specific antibodies against HBsAg, nine different serological subtypes were defined, reflecting the genetic variability of HBV. Of the defined determinants, one is common to all subtypes (a determinant), but also two pairs of mutually exclusive subdeterminants (d or y, and w or r) were commonly found. By using this tool in epidemiological studies, nine serological subtypes have been identified: ayw1, ayw2, ayw3, ayw4, ayr, adw2, adw4, adr_q⁺ and adr_q⁻ (Swenson et al., 1991).

Genotypically, HBV genomes have been classified into six groups, designated A-F, based on an intergroup divergence of 8% or more in the complete nucleotide sequence (Okamoto et al., 1988; Norder et al., 1992; Magnius and Norder, 1995). These six different genotypes show a characteristic geographical distribution: genotype A is pandemic, but most prevalent in north-west Europe, North America, and Central Africa; genotype B is mostly found in Indonesia, China and Vietnam; genotype C is found in East Asia, Korea, China, Japan, Polynesia and Vietnam; genotype D is also

more or less pandemic, but is predominant in the Mediterranean area and the Middle East extending to India; genotype E is endemic in Africa; and genotype F is found in American natives and in Polynesia (Van Geyt et al., 1998; Magnius & Norder, 1995). Some studies have shown that, in certain populations where HBV is endemic, a higher variability of HBV might be expected (Bowyer et al., 1997; Carman et al., 1997). In the present prevalence study of the HBV genotypes in France and the USA, HBV strains were identified that did not allow unique type recognition.

AIMS OF THE INVENTION

It is an aim of the present invention to provide new HBV polynucleic acid sequences that are unique to a heretofore unidentified HBV genotype, genotype G, which is phylogenetically different from HBV genotypes A, B, C, D, E and F.

It is another aim of the invention to provide fragments of the HBV polynucleic acids as described above, that are unique to the heretofore unidentified HBV genotype G and that contain at least one nucleotide or a combination of nucleotides different from known nucleic acid sequences of HBV genotypes A, B, C, D, E or F, or the complement of said fragments.

It is another aim of the present invention to provide fragments of the HBV polynucleic acids as described above that are universal to genotypes A, B, C, D, E, F and G.

It is another aim of the invention to provide a method for the detection in a biological sample of the presence of an HBV genotype G specific polynucleic acid.

It is another aim of the invention to provide a universal method for the detection of HBV genotypes A, B, C, D, E, F and G.

It is another aim of the invention to provide an oligonucleotide primer that is able to act as primer for specifically sequencing or specifically amplifying a nucleic acid or part of a nucleic acid characteristic to HBV genotype G.

It is another aim of the invention to provide a universal primer that is able to act as a primer for sequencing or amplifying nucleic acids from HBV genotypes A, B, C, D, E, F and G.

It is another aim of the invention to provide an oligonucleotide probe that is able to act as a hybridization probe for specific detection of a nucleic acid or part of a nucleic acid characteristic to HBV genotype G.

It is another aim of the invention to provide a universal probe that is able to act as a hybridization probe for the universal detection of nucleic acids from HBV genotypes A, B, C, D, E, F and G.

It is another aim of the invention to provide a method for HBV genotyping.

It is another aim of the invention to provide a diagnostic kit for the detection of a nucleic acid or part of a nucleic acid characteristic to HBV genotype G.

It is another aim of the invention to provide a diagnostic kit for the universal detection of HBV genotypes A, B, C, D, E, F and G.

It is another aim of the present invention to provide a HBV genotype G specific polypeptide.

- 5 It is another aim of the present invention to provide a universal peptide characteristic to genotypes A, B, C, D, E, F, and G.

It is another aim of the present invention to provide a method for the production of a polypeptide of the invention.

- 10 It is another aim of the present invention to provide an expression vector that allows the expression of a peptide of the invention.

It is another aim of the present invention to provide a host cell transformed with an expression vector of the invention.

- 15 It is another aim of the present invention to provide a method for the detection of antibodies that are specific to the heretofore unidentified HBV genotype or antibodies that recognize HBV genotypes A, B, C, D, E, F and G.

It is another aim of the present invention to provide a diagnostic kit for the detection of antibodies that are specific to the heretofore unidentified HBV genotype or for the detection of antibodies that recognize HBV genotypes A, B, C, D, E, F and G.

- 20 It is another aim of the present invention to provide an antibody or a ligand that specifically binds a peptide of the invention.

It is another aim of the present invention to provide an antibody or a ligand that specifically recognizes the HBV polymerase, X protein, preCore, HBcAg, HBeAg, preS1, preS2 or HBsAg protein of genotype G.

- 25 It is another aim of the present invention to provide an antibody or a ligand that recognizes the HBV polymerase, preCore, HBcAg, HBeAg, preS1, preS2 or HBsAg protein of genotypes A, B, C, D, E, F and G.

It is another aim of the present invention to provide a method for the detection of a polypeptide of the invention.

- 30 It is another aim of the present invention to provide a diagnostic kit that allows the detection of a polypeptide of the invention.

It is another aim of the present invention to provide a monoclonal antibody for the prevention or treatment of HBV infections.

It is another aim of the present invention to provide a vaccine or a pharmaceutical composition for the prevention or treatment of HBV infections.

It is another aim of the present invention to provide a method to identify compounds or agents that can be used for the prevention or treatment of HBV infections.

- 5 It is another aim of the present invention to provide an HBV genotype G infected clone for use in a drug-screening assay.

FIGURE LEGENDS

Figure 1. Complete nucleotide sequence of FR1 with amino acid translation of the four major ORFs. Target sequences for HBV genotype G specific probes and/or primers are indicated with an horizontal line. Nucleotides and nucleotide combinations that are unique to HBV genotype G are shown in bold and with a , respectively.

Figure 2. Complete coding sequence of the HBV preC/C gene as described by Tran et al. (1991).

Figure 3. Complete coding sequence of the HBV preS/S gene as described by Tran et al. (1991).

Figure 4. The genome organization and open reading frames of HBV genotype G virus compared to other genotypes. For each genotype, only one representative genome is included (genotype A: X70185; B: D00331; C: X01587; D: X72702; E: X75664; F: X75663; G: FR1). Positions in the viral genome where extensive variability is observed between the genotypes, are indicated as a black zone. Nucleotide and amino acid numbering is indicated. (1): Most isolates of genotype G contain translational stop codons, influencing the length of the preCore region. xxx: stands for the amino acid numbering for the M-residue in the conserved YMDD motif of the different genotypes. The positions and orientation of the primers shown in table 1 are indicated with arrows.

Figure 5. Amino acid sequence alignment of the preCore and Core regions of the different HBV genotypes. For each genotype, only one representative genome is included (genotype A: X70185; B: D00331; C: X01587; D: X72702; E: X75664; F: X75663; G: FR1). The amino acid sequence was derived from the nucleotide sequence. X: translational stop.

Figure 6. Amino acid sequence alignment of the preS1, preS2 and HBsAg open reading frame of the different HBV genotypes. For each genotype, only one

representative genome is included (genotype A: X70185; B: D00331; C: X01587; D: X72702; E: X75664; F: X75663; G: FR1). Top: preS1; middle: preS2; bottom: HBsAg. The amino acid sequence was derived from the nucleotide sequence.

- 5 **Figure 7.** Alignment of preCore/Core sequences of a HBV genotype A strain (HBVXCPS) and of 7 HBV strains that belong to the newly identified genotype G (FR1, FR2, US1, US3, US5, US6, US7, US10). N indicates the absence of a nucleotide at this position.
- 10 **Figure 8.** Alignment of preS1/preS2/HBsAg sequences of a HBV genotype A strain (HBVXCPS) and of 7 HBV strains that belong to the newly identified genotype G (FR1, FR2, US1, US3, US6, US7, US9, US10). N indicates the absence of a nucleotide at this position.
- 15 **Figure 9.** Phylogenetic distances, obtained by the program DNADIST, between the different HBV genotypes. In total 36 complete genomes (accession numbers are given in Fig. 10) were compared with the FR1 (genotype G) sequence. The mean for each group is indicated (A: ■; B: ●; C: ▲; D: X; E: *; F: ◆; G: +), including the standard error of the mean; the latter gives a 95% confidence interval around these distances.
- 20 **Figure 10.** Phylogenetic trees of the HBV genotypes. Virus isolates are indicated by their GenBank accession number. (a) Complete genomes. (b) Open reading frame of the surface gene (including preS1/preS2/HBsAg).
- 25 **Figure 11.** Result obtained with some representative samples of genotypes A, B, C, D, E, F and G after hybridization of their PCR product to the genotyping LiPA as designed in example 4. Strip G: incubated with PCR fragments of FR1. The right strip shows the positions of all probes applied. The numbering of the probes is described in Van Geyt et al. (1998).
- 30 Conj.contr: conjugate control; Ampl.contr: amplification control (probe reacting with all genotypes, located in conserved area). The probe lines with "target A" and "target B" contain probes recognizing the target A and target B region as indicated in Figure 1. Probes 193 and 77 recognize genotype A only. Probe 78 recognizes genotype B

only. Probes 153, 154 and 204 recognize genotype C only. Probes 165 and 208 recognize genotype D only. Probes 172, 177 and 213 recognize genotype E only. Probes 186, 216, and 219 recognize genotype F only. Probe 140 recognizes genotypes A and G. Probe 148 recognizes genotypes A, B and G. Probe 80 recognizes genotypes C, D, E and G. Probe 239 recognizes genotypes B, E and G.

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Figure 12. Complete coding sequence of the HBV preC/C gene as described by Bhat et al. (1990).

TABLES

Table 1. Overview of the amplification and sequencing primers.

Primers	Primer sequence 5' - 3'	Polarity	Domain	Position	SEQ ID NO
HBPt 1	GGGTACCATATTCTTGGG	S	HBPoI	2850-2868	21
HBPt 2	GAACAAGAGCTACAGCATGGG	S	HBPoI/PreS1	2867-2888	22
HBPt 3	CCACTGCATGGCCTGAGGATG	AS	PreS1/S2/HBPoI	3226-3246	23
HBPt 7	CTCCACAG(A/T)AGCTCCAAATTC	AS	Core	1958-1978	24
HBPt 14	TGGGGTGGAGCCCTCAG	S	PreS1/HBPoI	3104-3120	25
HBPt 33	CTGAGGGCTCCACCCCA	AS	PreS1/HBPoI	3104-3120	26
HBPt 75	CAAGGTATGTGGCCGTTGTCC	S	HBsAg	455-477	27
HBPt 86	ACATAAGAGGACTCTTGAC	S	HBX	1652-1671	28
HBPt 87	TACTTCAAAGACTGTGTGTTA	S	HBX	1704-1723	29
HBPt 94	GGTA(A/T)AAAGGGACTCA(C/A)GATG	AS	HBPoI/HBsAg	775-795	30
HBPt 108	TTTTTCACCTCTGCCTAATC	S	HBX/PreCore	1821-1840	31
HBPt 109	AAAAAGTTGCATGGTGCTGG	AS	HBX/PreCore	1806-1825	32
HBPt 110	CCTCTGCCGATCCATACTGCGGAAC	S	HBPoI	1255-1279	33
HBPt 111	CTGCGAGGCGAGGGAGTTCTTCTC	AS	Core/HBPoI	2406-2430	34
HBPt 113	CCGGCAGATGAGAAAGGCACAGACGG	AS	HBX/HBPoI	1549-1574	35
HBPt 134	TGCTGCTATGCCTCATCTTC	S	HBPoI/HBsAg	414-433	36

Table 1. Continued.

Primers	Primer sequence 5' - 3'	Polarity	Domain	Position	SEQ ID NO
HBPt 135	CA(A/G)AGACAAAAGAAAATTGG	AS	HBPoI/HBsAg	803-822	37
HBPt 303	CCCACCTTATGAGTCCAAGG	AS	HBPoI	2493-2512	38
HBPt 374	GTTCGCGAGTATGGATCGGCAGAGG	AS	HBPoI	1255-1279	39
HBPt 440	TATGGATGATGTGGTATTGGG	S	HBPoI/HBsAg	738-758	40
HBPt 446	GGAGTGTGGATTCGCACTCC	S	Core	2303-2323	41
HBPt 448	CCCATGCTGTAGCTCTTGTTTC	AS	HBPoI PreS1	2868-2888	42

S = sense primer; AS = antisense primer.

Table 2. Summary of the genotyping result obtained from 121 individuals infected with HBV.

Genotype	A	B	C	D	E	F	G	Total
France	18	0	2	16	1	0	2	39
Georgia,	48	4	12	7	0	0	11	82
U.S.								
Total	66(54%)	4(3%)	14(12%)	23(19%)	1(1%)	0(0%)	13(11%)	121(100%)

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to an isolated and/or purified HBV polynucleic acid, having a nucleotide sequence which is unique to a heretofore unidentified HBV genotype and which is phylogenetically different from HBV genotypes A, B, C, D, E and F characterized by:

- (a) a sequence as defined in Figure 1 (SEQ ID NO 1); or
- (b) a sequence with at least 90%, more preferably 91%, most preferably 92% identity to the sequence as defined in Figure 1; or
- (c) a sequence which is degenerate as a result of the genetic code to the sequence as defined in (a) and (b).

An "individual genetic group" or "phylogenetically different genotype" of HBV can be defined if a certain viral strain differs by more than 8% from all other HBV genomes (Okamoto et al., 1988; Norder et al., 1994). Thus, virus strains differing from other isolates by more than 8% over their entire genome (not focussing on possible deletions or insertions) are classified as new genotypes. Based on results obtained from several epidemiological studies (Blitz et al., 1998; Norder et al., 1994; Magnius and Norder, 1995; Telenta et al., 1997; Alvarado-Esquivel et al., 1998), six major genetic groups of HBV (A, B, C, D, E and F) were recognized. Apart from the detection of these commonly found genotypes, in a prevalence study on samples from chronic HBV carriers from Atlanta, USA and Lyon, France, the present inventors have isolated a new viral HBV strain with at least 11.7% (new genotype versus genotype E) and at most 15.3% (new genotype versus genotype F) divergence over the complete genome. Accordingly, this new viral HBV strain has been classified as a new HBV genotype G. Surprisingly, the prevalence of this viral variant in the US exceeded 11% of all infections, although it had not been identified in all of the previous molecular epidemiology studies. In addition to the prominent prevalence of this new genotype G virus in the USA samples, it was also found in samples originating from France.

The term "isolated" refers to material that is free from components that normally accompany it as found in its naturally occurring environment. However, it should be clear that the isolated nucleic acid of the present invention might comprise heterologous cell components or a label and the like.

The terms "nucleic acid" or "polynucleic acid" are used interchangeable throughout the present application and refer to a deoxyribonucleotide or ribonucleotide polymer in either single- or double stranded form, which may encompass known analogues of natural nucleotides that hybridize to nucleic acids in a manner similar to naturally occurring nucleotides. The term specifically refers to the DNA sequence as defined in Figure 1 and given by SEQ ID NO 1 or the complementary strand thereof. Also within the scope of the present invention are all HBV nucleic acids that belong to the new HBV genotype G and that thus differ 10%, more preferably 9%, most preferably 8% or less from the nucleotide sequence as depicted in Figure 1 or that are at least 90%, more preferably 91%, most preferably 92% identical to the sequence as depicted in Figure 1.

A nucleic acid sequence that "differs 10%, 9%, 8% or less from another nucleic acid sequence" is a nucleic acid sequence of which respectively 10%, 9%, 8% or less of its total nucleotides are different from the nucleotides of the nucleic acid sequence it is compared with.

A nucleic acid sequence that "is at least 90%, 91%, or 92% identical to another nucleic acid sequence" is a nucleic acid sequence of which respectively at least 90%, 91%, or 92% of its total nucleotides are identical to the nucleotides of the nucleic acid sequence it is compared with.

As a result of the genetic code, also nucleic acid sequences that show less than 90%, more preferably 91%, most preferably 92% identity to the sequence as depicted in Figure 1 can encode the same proteins as is encoded by the nucleotide sequences of HBV strains belonging to the new genotype G. Therefore also these nucleic acid sequences, that are degenerate as a result of the genetic code, fall within the scope of the present invention.

In contrast to the known HBV genotypes, the complete genome of the HBV sequence of the invention, belonging to the newly identified genotype G, was found to be 3248 bp long (Fig. 4). The preCore region has translational stops at codon 2 (TAA instead of CAA) and codon 28 (TAG instead of TGG) (Fig. 5). The Core region is 585 nucleotides long, encoding a Core protein of 195 amino acids, and has a nucleotide insert of 36 bp, located after the fifth nucleotide following the Core translation initiation point (A at position 1901). Similar to all other genotypes except genotype A, the HBV sequence belonging to genotype G showed a 6 nucleotide deletion in the

carboxy-terminal part of the HBcAg ORF (Figs. 4 and 5). The preS1 region contains 354 bp (118 amino acids), the preS2 region 165 bp (55 amino acids) and the HBsAg region 678 bp (226 amino acids) (Fig. 6). Like genotype E, the HBV sequence belonging to genotype G showed a 3 nucleotide deletion (1 amino acid at position 11;

5 Fig. 6) in the amino-terminal part of preS1.

In accordance, the present invention specifically relates to an HBV polynucleic acid as described above, further characterized that its nucleotide sequence differs from HBV genotype A, B, C, D, E or F in at least one of the following characteristics:

- (a) it has a length of 3248 basepairs;
- 10 (b) it encodes a translational stop codon at amino acids 2 and 28 of the preCore region;
- (c) it has a 36 nucleotide insert in the N-terminal part of the Core region;
- (d) it has a 6 nucleotide deletion in the C-terminal part of the HBcAg ORF;
- (e) it has a 3 nucleotide deletion in the N-terminal part of the preS1 ORF.

15 In this specific embodiment, the HBV polynucleic acid sequence of the invention thus has 1, 2, 3, 4 or 5 of the above mentioned characteristics.

In a more specific embodiment, the present invention relates to an HBV polynucleic acid as described above, comprising at least one of the following sequences (Figs. 7 and 8): SEQ ID NO 44, SEQ ID NO 45, SEQ ID NO 46, SEQ ID NO 47, SEQ ID NO
20 48, SEQ ID NO 49, SEQ ID NO 50, SEQ ID NO 51, SEQ ID NO 53, SEQ ID NO 54, SEQ ID NO 55, SEQ ID NO 56, SEQ ID NO 57, SEQ ID NO 58, SEQ ID NO 59, SEQ ID NO 60.

The present invention further relates to a fragment of a polynucleic acid as described above characterized that said fragment is unique to the heretofore unidentified HBV
25 genotype G and that said fragment contains at least one nucleotide or a combination of nucleotides different from known HBV genotypes A, B, C, D, E or F nucleotide sequences, or the complement of said fragment, provided that said fragment is not equal to the preC/C gene sequence as depicted in Figure 2 (SEQ ID NO 7) or the preS/S gene sequence as depicted in Figure 3 (SEQ ID NO 8).

30 Tran et al. (1991) have described the preS/S and preC/C sequences of an HBV recombinant strain which accidentally differ less than 8% from the preS/S and preC/C sequences of the strains of the present invention. In contrast to the newly identified HBV genotype G of the present invention, however, this atypical HBV strain was

described in the context of an event of emergence of and take over by HBV, with rearrangements in the preS/S and preC/C genes (Tran et al., 1991). This atypical HBV strain was never recognized as a new genotype. In order to study the genotype G sequence with respect to such recombination events (as described by Tran et al. 1991),
5 the present inventors have inspected the complete FR1 genome for such events on the nucleotide level, and phylogenetically for co-segregation with other known genotypes. Evidence for recombination was not found. The absence of recombination events in the newly isolated strain and the high prevalence of this new viral variant indicate that it concerns a new HBV genotype. The accidentally homologous HBV sequences
10 described by Tran et al. (1991) which have emerged only as a result of a recombination event, are disclaimed from the present invention. In accordance, the HBV preC/C gene sequence as shown in Figure 2 (SEQ ID NO 7; Accession number M74501; Tran et al. 1991) and the preS/S gene sequence as shown in Figure 3 (SEQ ID NO 8; Accession number M74499; Tran et al. 1991) do not form part of the
15 present invention. In the same respect, the HBV preC/C gene sequence as shown in Figure 12 (SEQ ID NO 169; Bhat et al. 1990) does not form part of the present invention and is therefore disclaimed. The HBV preC/C sequence by Bhat et al was disclosed and discussed in the context of a chronic carrier who lacked anti-HBc but carried exceedingly high levels of HBV DNA in serum. As for Tran et al (1991), the
20 link with the presence of a new genotype was never made.

The present invention also relates to a fragment of a polynucleic acid as described above characterized that said fragment is a universal fragment which is comprised in nucleic acid sequences of HBV genotypes A, B, C, D, E, F and G. Since the present invention provides nucleic acid alignments of 7 genotypes, the invention allows for
25 the first time to delineate universal HBV nucleic acids sequences, characteristic for HBV genotypes A, B, C, D, E, F and G.

The term "universal" as used in the present application means that it is present in, corresponds to, or is characteristic to HBV genotypes A, B, C, D, E, F, and G.

The fragment of the invention can be single stranded or double stranded. It can be
30 from 5 to 3247 bp long. More preferably this fragment is from 5 to 2600 bp long, from 5 to 1300 bp long, from 5 to 700 bp long, from 5 to 500 bp long, from 5 to 300 bp long and most preferably from 5 to 200 bp long. The fragment can be used for

cloning and expression or the fragment can be used as primer or a probe in an HBV typing method.

The present invention therefore also relates to an oligonucleotide primer comprising a fragment as described above, with said primer being a HBV genotype G specific primer, able to act as primer for specifically sequencing or specifically amplifying a nucleic acid or part of a nucleic acid characteristic to HBV genotype G. The present invention also relates to an oligonucleotide primer comprising a fragment as described above, with said primer being a universal primer, able to act as primer for sequencing or amplifying nucleic acids from HBV genotypes A, B, C, D, E, F and G. The term "primer" refers to a single stranded oligonucleotide sequence capable of acting as a point of initiation for synthesis of a primer extension product that is complementary to the nucleic acid strand to be copied. The length and the sequence of the primer must be such that they allow to prime the synthesis of the extension products. Preferably, the length of the primer is about 5-50 nucleotides. More preferably, the length of the primer is about 10-30 nucleotides. Most preferably, the length of the primers is about 20-25 nucleotides. Specific length and sequence will depend on the complexity of the required DNA or RNA target, as well as on the conditions at which the primer is used, such as temperature and ionic strength.

The present invention also relates to an oligonucleotide probe comprising a fragment as described above, with said probe being a HBV genotype G specific probe, able to act as a hybridization probe for the specific detection of a nucleic acid or part of a nucleic acid characteristic to HBV genotype G. The present invention also relates to an oligonucleotide probe comprising a fragment as described above, with said probe being a universal probe, able to act as a hybridization probe for nucleic acids from HBV genotypes A, B, C, D, E, F and G. The term "probe" refers to a single stranded sequence-specific oligonucleotide that has a sequence that is complementary to the target sequence in the HBV genome. Preferably, these probes are about 5 to 50 nucleotides long, more preferably from about 10 to 25 nucleotides. Particularly preferred lengths of probes include 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 nucleotides. The nucleotides used in the probes of the present invention may be ribonucleotides, deoxyribonucleotides and modified nucleotides such as inosine, or nucleotides containing modified groups that do not essentially alter their hybridization characteristics.

The probes and/or primers according to the invention can be prepared by cloning recombinant plasmids containing inserts including the corresponding nucleotide sequence, if needed followed by excision of the latter from the cloned plasmids by use of the adequate nucleases and recovery of the primers and/or probes, e.g. by
5 fractionation according to molecular weight. The primers and/or probes according to the present invention can also be synthesized chemically, for instance by the conventional phospho-triester method. They can also be part of a branched DNA capture or detection probe (Sanchez-Pescador et al., 1988; Urdea et al., 1991)

The primers and/or probes of the invention may be labeled. Labeling may be carried
10 out by any method known to the person skilled in the art. The nature of the label may be isotopic (^{32}P , ^{35}S , etc.) or non-isotopic (biotin, digoxigenin, etc.) or may reside on a branched DNA probe.

The oligonucleotides used as primers and/or probes may also contain or consist of nucleotide analogues such as phosphorothiates (Matsukura et al., 1987),
15 alkylphosphorothiates (Miller et al., 1979) or peptide nucleic acids (Nielsen et al., 1991; Nielsen et al., 1993) or may contain intercalating agents (Asseline et al., 1984). The introduction of these modifications may be advantageous in order to positively influence characteristics such as hybridization kinetics, reversibility of the hybrid-formation, biological stability of the oligonucleotide molecules, etc.

20 As most other variations or modifications introduced into the original DNA sequences of primers and/or probes, these variations will necessitate adaptations with respect to the conditions under which the oligonucleotide should be used to obtain the required specificity and sensitivity. The eventual results of the priming or hybridization with these modified oligonucleotides, however, should be essentially the same as those
25 obtained with the unmodified oligonucleotides.

In a preferred embodiment of the invention, the probe and/or primer recognizes one of the target sequences as indicated in Figure 1.

The term "target sequence" of a probe and/or primer, according to the present invention, is a sequence within the genome of the newly identified genotype G that
30 comprises one or more nucleotides that are unique on their own or as a combination to the newly identified genotype G and to which the probe or primer is complementary or partially complementary (i.e. with up to 20%, more preferably 15%, more

preferably 10% or most preferably 5% mismatches). It is to be understood that the complement of said target sequence is also a suitable target sequence in some cases.

It should be understood that probes that are designed to specifically hybridize to a target sequence of a nucleic acid, may fall within said target sequence or may to a large extent overlap with said target sequence (i.e. form a duplex with nucleotides outside as well as within said target sequence).

In a very specific embodiment of the invention the target sequence is chosen within the 36 nucleotide insert in the N-terminal part of the Core region of HBV genotype G:

10 TAGAACAACCTTTGCCATATGGCCTTTTTGGCTTAGA (SEQ ID NO 61)

More specifically, the primer and/or probe of the invention recognizes one or more of the following sequences:

15 TAGAA (SEQ ID NO 62);
AGAAC (SEQ ID NO 63);
GAACA (SEQ ID NO 64);
AACAA (SEQ ID NO 65);
ACAAC (SEQ ID NO 66);
20 CAACT (SEQ ID NO 67);
AACTT (SEQ ID NO 68);
ACTTT (SEQ ID NO 69);
CTTTG (SEQ ID NO 70);
TTTGC (SEQ ID NO 71);
25 TTGCC (SEQ ID NO 72);
TGCCA (SEQ ID NO 73);
GCCAT (SEQ ID NO 74);
CCATA (SEQ ID NO 75);
CATAT (SEQ ID NO 76);
30 ATATG (SEQ ID NO 77);
TATGG (SEQ ID NO 78);
ATGGC (SEQ ID NO 79);
TGGCC (SEQ ID NO 80);

GGCCT (SEQ ID NO 81);
GCCTT (SEQ ID NO 82);
CCTTT (SEQ ID NO 83);
CTTTT (SEQ ID NO 84);
5 TTTTT (SEQ ID NO 85);
TTTTG (SEQ ID NO 86);
TTTGG (SEQ ID NO 87);
TTGGC (SEQ ID NO 88);
TGGCT (SEQ ID NO 89);
10 GGCTT (SEQ ID NO 90);
GCTTA (SEQ ID NO 91);
CTTAG (SEQ ID NO 92);
TTAGA (SEQ ID NO 93);
TAGAAC (SEQ ID NO 94);
15 AGAACA (SEQ ID NO 95);
GAACAA (SEQ ID NO 96);
AACAAC (SEQ ID NO 97);
ACAAC (SEQ ID NO 98);
CAACTT (SEQ ID NO 99);
20 AACTTT (SEQ ID NO 100);
ACTTTG (SEQ ID NO 101);
CTTTGC (SEQ ID NO 102);
TTTGCC (SEQ ID NO 103);
TTGCCA (SEQ ID NO 104);
25 TGCCAT (SEQ ID NO 105);
GCCATA (SEQ ID NO 106);
CCATAT (SEQ ID NO 107);
CATATG (SEQ ID NO 108);
ATATGG (SEQ ID NO 109);
30 TATGGC (SEQ ID NO 110);
ATGGCC (SEQ ID NO 111);
TGGCCT (SEQ ID NO 112);
GGCCTT (SEQ ID NO 113);

GCCTTT (SEQ ID NO 114);
CCTTTT (SEQ ID NO 115);
CTTTTT (SEQ ID NO 116);
TTTTTG (SEQ ID NO 117);
5 TTTTGG (SEQ ID NO 118);
TTTGGC (SEQ ID NO 119);
TTGGCT (SEQ ID NO 120);
TGGCTT (SEQ ID NO 121);
GGCTTA (SEQ ID NO 122);
10 GCTTAG (SEQ ID NO 123);
CTTAGA (SEQ ID NO 124);
TAGAACA (SEQ ID NO 125);
AGAACAA (SEQ ID NO 126);
GAACAAC (SEQ ID NO 127);
15 AACAACT (SEQ ID NO 128);
ACAACCT (SEQ ID NO 129);
CAACTTT (SEQ ID NO 130);
AACTTTG (SEQ ID NO 131);
ACTTTGC (SEQ ID NO 132);
20 CTTTGCC (SEQ ID NO 133);
TTTGCCA (SEQ ID NO 134);
TTGCCAT (SEQ ID NO 135);
TGCCATA (SEQ ID NO 136);
GCCATAT (SEQ ID NO 137);
25 CCATATG (SEQ ID NO 138);
CATATGG (SEQ ID NO 139);
ATATGGC (SEQ ID NO 140);
TATGGCC (SEQ ID NO 141);
ATGGCCT (SEQ ID NO 142);
30 TGGCCTT (SEQ ID NO 143);
GGCCTTT (SEQ ID NO 144);
GCCTTTT (SEQ ID NO 145);
CCTTTTTT (SEQ ID NO 146);

CTTTTGTG (SEQ ID NO 147);
TTTTTG (SEQ ID NO 148);
TTTTGGC (SEQ ID NO 149);
TTTGGCT (SEQ ID NO 150);
5 TTTGGCTT (SEQ ID NO 151);
TGGCTTA (SEQ ID NO 152);
GGCTTAG (SEQ ID NO 153);
GCTTAGA (SEQ ID NO 154);

- 10 The invention also relates to the use of a HBV genotype specific primer and/or a probe of the invention in a method to detect, in a biological sample, the presence of the new HBV genotype G.

The invention also relates to the use of a universal primer and/or a probe of the invention in a universal method to detect, in a biological sample, the presence of
15 HBV.

The present invention further relates to a method for the detection in a biological sample of the presence of a polynucleic acid characteristic to HBV genotype G.

The present invention further relates to a method for HBV genotyping using a primer and/or probe of the present invention.

- 20 More specifically, the present invention relates to a method as described above, further characterized that it comprises the following step:

- (i) possibly extracting the nucleic acid present in the biological sample;
- (ii) amplifying the nucleic acid characteristic to HBV genotype G;
- (iii) detecting the amplified nucleic acid characteristic to HBV genotype G.

- 25 Primers used for amplification may be generic primers or genotype G specific primers. Generic primers allow the amplification of DNA from strains that belong to genotype G as well as DNA from other strains belonging to other HBV genotypes. Genotype G specific primers, in contrast, will allow only the amplification of the nucleic acids from strains that belong to genotype G. These genotype G specific
30 primers also fall within the scope of the present invention.

The present invention further relates to a method for the universal detection in a biological sample of the presence of HBV.

More specifically, the present invention relates to a universal method as described above, further characterized that it comprises the following step:

- (i) possibly extracting the nucleic acid present in the biological sample;
- (ii) amplifying the nucleic acid;
- 5 (iii) detecting the amplified nucleic acid.

Primers used for amplification in said universal method are universal primers that allow the amplification of DNA from all genotypes A, B, C, D, E, F and G.

The term "biological sample" as used in the present invention refers to any biological material (tissue or fluid) taken either directly from the infected human being, or after
10 culturing (enrichment) and containing HBV nucleic acid sequences. Biological material may be e.g. expectoration's of any kind, broncheolavages, blood, skin tissue, biopsies, sperm, lymphocyte blood culture material, colonies, liquid cultures, fecal samples, urine, hepatocytes, etc. More particularly "biological sample" refers to blood serum or plasma samples.

15 The nucleic acids are released, concentrated and/or isolated from the biological sample by any method known in the art. Currently, various commercial kits are available such as the QIAamp Blood Kit from Qiagen (Hilden, Germany) for the isolation of nucleic acids from blood samples and the 'High pure PCR Template Preparation Kit' (Roche Diagnostics, Brussels, Belgium). Other well-known
20 procedures for isolation of DNA or RNA from a biological sample are also available (Sambrook et al., 1989).

The nucleic acids of the invention can be amplified by polymerase chain reaction (PCR; Saiki et al., 1988), ligase chain reaction (LCR; Landgren et al., 1988; Wu and Wallace, 1989; Barany, 1991), nucleic acid sequence-based amplification (NASBA;
25 Guatelli et al., 1990; Compton, 1991), transcription-based amplification system (TAS; Kwoh et al., 1989), strand displacement amplification (SDA; Duck, 1990) or amplification by means of Q β replicase (Lomeli et al., 1989) or by any other suitable method known in the art, that allows the amplification of nucleic acid molecules. Also TMA (Guatelli et al., 1990) or bDNA (Sanchez-Pescador et al., 1988; Urdea et al.,
30 1991) techniques can be used in the method of the present invention.

The products of this amplification step can subsequently be used for detection of the presence, and/ or for typing of the nucleic acids present in the sample. Analysis of the presence and typing of these nucleic acids can be done by any method known in the

art, such as duplex analysis of the PCR products (Clay et al., 1994), single-stranded conformational polymorphism analysis of the PCR product (PCR-SSCP; Yoshida et al., 1992), sequence-based typing (SBT; Santamaria et al., 1992 and 1993), the use of sequence specific primers in PCR reaction (PCR-SSP; Olerup and Zetterquist, 1991),
5 the use of PCR in combination with sequence-specific oligonucleotide probing (PCR-SSOP; Saiki et al., 1986), TMA or bDNA techniques. Sequencing can be performed via any known sequencing method such as the enzymatic dideoxy method of Sanger et al (1977) or the chemical method of Maxam and Gilbert (1977, 1980). Kits and/or tools for thermal-cycle sequencing, solid-phase sequencing and automated sequencing
10 are commercially available. Recent sequencing techniques provide for simultaneous sequencing in the 5' and 3' directions. Some examples of automated sequencers are the MicroGene ClipperTM 2 Dye and the MicroGene BlasterTM from the OpenGeneTM system (Visible Genetics Inc., Toronto, Ontario, Canada) and the ABI PRISM[®] system (Perkin Elmer Inc., PE Biosystems, PE Applied Biosystems, Foster City,
15 California, USA). Assay methods that rely on the formation of a hybrid between the nucleic acids in the biological sample and the oligonucleotide probe include Southern blot, Northern blot or dot blot format (Saiki et al., 1989), the unlabelled amplified sample being bound to a membrane, the membrane being incorporated with at least one labeled probe under suitable hybridization and wash conditions, and the presence of
20 bound probe being monitored. An alternative is a "reverse" format, in which the amplified sequence contains a label. In this format, the selected probes are immobilized to certain locations on a solid support and the amplified polynucleic acids are labeled in order to enable the detection of the hybrids formed. The term "solid support" can refer to any substrate to which an oligonucleotide probe can be coupled, provided that
25 it retains its hybridization characteristics and provided that the background level of hybridization remains low. Usually the solid substrate will be a microtiter plate (e.g. in the DEIA technique), a membrane (e.g. nylon or nitrocellulose) or a microsphere (bead) or a chip. Prior to application to the membrane or fixation it may be convenient to modify the nucleic acid probe in order to facilitate fixation or improve the
30 hybridization efficiency. Such modifications may encompass homopolymer tailing, coupling with different reactive groups such as aliphatic groups, NH₂ groups, SH groups, carboxylic groups, or coupling with biotin, haptens or proteins.

A preferred embodiment of the present invention thus relates to a method as described above, further characterized that it comprises the following step:

- (i) possibly extracting the nucleic acid present in the biological sample;
- (ii) amplifying the nucleic acid;
- 5 (iii) hybridizing the nucleic acid, possibly under denatured conditions, at appropriate conditions with one or more probes, with said probes being possibly attached to a solid substrate;
- (iv) possibly washing at appropriate conditions;
- (v) detecting the hybrids formed.

10 In order to obtain fast and easy results, if a multitude of probes is involved, a reverse hybridization format may be convenient. In this preferred embodiment the selected probes are immobilized to certain locations on a solid support and the amplified polynucleic acids are labeled in order to enable the detection of the hybrids formed.

The present invention further relates to a diagnostic kit for use in a method as
15 described above, comprising the following components:

- (i) when appropriate, a means for releasing, isolating or concentrating the nucleic acids present in the sample;
 - (ii) a primer set or a primer mix, possibly according to the invention;
 - (iii) a means for detection or typing of the nucleic acid, present in the sample.
- 20 The present invention specifically relates to a diagnostic kit as described above, said kit comprising at least one primer and/or at least one probe of the invention. A specific and very user-friendly diagnostic kit is the line probe assay (Stuyver et al., 1996), comprising the following components:

- (i) when appropriate, a means for releasing, isolating or concentrating the
25 nucleic acids present in said sample;
- (ii) a primer pair or a primer mix, possibly according to the invention;
- (iii) at least one probe that specifically hybridizes with a nucleic acid characteristic to HBV genotype G, or at least one universal probe, fixed to a solid support;
- 30 (iv) a hybridization buffer, or components necessary for producing said buffer;
- (v) a wash solution, or components necessary for producing said solution;

(vi) when appropriate, a means for detecting the hybrids resulting from the preceding hybridization.

In this embodiment, the selected set of probes is immobilized to a membrane strip in a line fashion. Said probes may be immobilized individually or as mixtures to the delineated locations. The amplified HBV polynucleic acids can be labeled with biotin, and the hybrid can then, via a biotin-streptavidine coupling, be detected with a non-radioactive color developing system.

The term "hybridization buffer" means a buffer allowing a hybridization reaction between the probes and the polynucleic acids present in the sample, or the amplified products, under the appropriate stringency conditions.

The term "wash solution" means a solution enabling washing of the hybrids formed under the appropriate stringency conditions.

The invention also relates to a peptide or a polypeptide having an amino acid sequence encoded by a polynucleic acid of the invention. The terms "peptide" and "polypeptide" are used interchangeable throughout the present application and refer to a polymer of amino acids (aa) and do not refer to a specific length of the product. Thus, oligopeptides, polypeptides, peptides, proteins and precursors are included within this definition. More particularly, the polypeptide of the present invention is a HBV genotype G specific polypeptide such as the preS1, preS2, surface antigen HBsAg, preCore precursor protein, HBeAg, HBcAg, the polymerase or the X protein of the HBV strains belonging to the newly identified genotype G, or any part thereof or any combination thereof, provided that said polypeptide differs in at least one amino acid from known polypeptides from HBV strains belonging to genotypes A, B, C, D, E or F. In a very specific embodiment, the present invention relates to the HBcAg or HBeAg of the HBV genotype G or any part thereof. The insert in the core region, encoding twelve additional amino acids RTTLPYGLFGLD (SEQ ID NO 155) may be responsible for a novel mechanism of expression of HBcAg and HBeAg, and core and e antigens with a unique structure and immunological properties may exist. Therefore, the present invention specifically relates to a peptide derived from the HBcAg or HBeAg of HBV genotype G comprising one of the following sequences:

RTTLPY (SEQ ID NO 156);

TTLPYG (SEQ ID NO 157);
TLPYGL (SEQ ID NO 158);
LPYGLF (SEQ ID NO 159);
PYGLFG (SEQ ID NO 160);
5 YGLFGL (SEQ ID NO 161);
GLFGLD (SEQ ID NO 162);

RTTLPYG (SEQ ID NO 163);
TTLPYGL (SEQ ID NO 164);
10 TLPYGLF (SEQ ID NO 165);
LPYGLFG (SEQ ID NO 166);
PYGLFGL (SEQ ID NO 167);
YGLFGLD (SEQ ID NO 168);

15 The present invention further relates to a universal peptide, characterized that said peptide is comprised in peptide sequences of HBV genotypes A, B, C, D, E, F and G. Since the present invention provides amino acid sequence alignments of 7 genotypes, the invention allows for the first time to delineate universal HBV amino acid sequences, characteristic for HBV genotypes A, B, C, D, E, F and G.

20 Also included within the present invention are post-translational modifications such as glycosylation, acetylation, phosphorylation, modifications with fatty acids and the like. Also included within the definition are, for example, polypeptides containing one or more analogues of an aa (including unnatural amino acids), polypeptides with substituted linkages, mutated versions or natural sequence variations of the
25 polypeptides, polypeptides containing disulfide bounds between cysteine residues, biotinylated polypeptides as well as other modifications known in the art.

The polypeptides of the invention can be produced by any method known in the art such as classical chemical synthesis as described by Houbenweyl (1974) and Atherton and Shepard (1989) or by means of recombinant DNA techniques as described by
30 Sambrook et al. (1989).

In accordance, the present invention also relates to a polypeptide as described above which is recombinantly expressed. The term "recombinantly expressed" refers to the fact that the peptide is produced by recombinant expression methods be it in

prokaryotes, or lower or higher eukaryotes as discussed below. The present invention thus also relates to a method for the production of a recombinant polypeptide of the invention comprising the following steps:

- (i) transformation of an appropriate cellular host with an expression vector;
- 5 (ii) culturing said transformed cellular host under conditions enabling the expression of the polynucleic acid inserted in the expression vector; and
- (iii) harvesting said polypeptide.

Therefore, also comprised within the scope of the present invention is an expression vector, comprising a polynucleic acid or part thereof of the invention, operably linked
10 to prokaryotic, eukaryotic or viral transcription and translation control elements. The term "expression vector" refers to a vector sequence of the type plasmid, cosmid, phage or viral DNA wherein a nucleic acid of the invention is inserted, and which contains the necessary elements to promote the transcription and translation of the latter polypeptide(s) by a host cell.

15 The present invention also relates to a host cell transformed with an expression vector as defined above. The term "host cell" denotes micro-organisms or higher eukaryotic cell lines cultured as unicellular entities and refers to cells which can be or have been used as recipients for a recombinant vector. The prokaryotes used as host cell can be chosen from bacteria such as *Escherichia coli*, *Lactobacillus*, *Lactococcus*,
20 *Salmonella*, *Streptococcus*, *Bacillus* or *Streptomyces*. Preferred lower eukaryotic host cells are yeasts, particularly species within *Saccharomyces*, *Schizosaccharomyces*, *Kluyveromyces*, *Pichia* (e.g. *Pichia pastoris*), *Hansenula* (e.g. *Hansenula polymorpha*), *Yarrowia*, *Schwanniomyces*, *Zygosaccharomyces* and the like. *Saccharomyces cerevisiae*, *Saccharomyces carlsbergensis* and *K. lactis* are the most
25 commonly used yeast hosts. Fungal host cells include *Neurospora crassa*. Host cells from higher eukaryotes include those from higher animals such as mammals, reptiles, insects, and the like. Presently, preferred higher eukaryotic host cells are derived from Chinese hamster (e.g. CHO), monkey (e.g. COS and Vero cells), baby hamster kidney (BHK), pig kidney (PK15), rabbit kidney 13 cells (RK13), the human osteosarcoma
30 cell line 143 B, the human cell line HeLa and human hepatoma cell lines like Hep G2, and insect cell lines (e.g. *Spodoptera frugiperda*). The host cells may be provided in suspension as flask cultures, tissue cultures, organ cultures and the like.

Any of the known purification methods for recombinant peptides can be used for the production of the recombinant peptides of the present invention.

The present invention also relates to the use of a HBV genotype G specific polypeptide of the invention in a method for detecting antibodies specific to the
5 heretofore unidentified HBV genotype G, preferably specific to the HBcAg and/or HBeAg of the heretofore unidentified HBV genotype G present in a biological sample.

The present invention also relates to a method for detecting antibodies specific to the heretofore unidentified HBV genotype G, preferably specific to the HBcAg and/or
10 HBeAg of the heretofore unidentified HBV genotype G present in a biological sample, comprising the following steps:

- (i) contacting the biological sample with a HBV genotype G specific polypeptide according to the invention;
- (ii) detecting the immunological complex formed between said antibodies
15 and said polypeptide.

The present invention also relates to a universal method for detecting antibodies specific to genotypes A, B, C, D, E, F and G present in a biological sample, comprising the following steps:

- (i) contacting the biological sample with a universal polypeptide according
20 to the invention;
- (ii) detecting the immunological complex formed between said antibodies and said polypeptide.

The present invention also relates to a universal method for HBV genotyping of a biological sample, comprising the following steps:

- (i) contacting the biological sample with at least one polypeptide according
25 to the invention;
- (ii) detecting the immunological complex formed between said antibodies and said polypeptide.

Design of the immunoassay is subject to a great deal of variation, and many formats
30 are known in the art. Protocols may, for example, use solid supports, or immunoprecipitation. Most assays involve the use of labeled polypeptides. The label may be, for example, enzymatic, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays that amplify the signals from the immune complex include the use

of biotin and avidin or streptavidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

The immunoassay may be, without limitation, in a heterogeneous or in a homogeneous format, and of a standard or competitive type. In a heterogeneous format, the polypeptide is typically bound to a solid matrix or support to facilitate separation of the sample from the polypeptide after incubation. Examples of solid supports that can be used are nitrocellulose (e.g., in membrane or microtiter well form), polyvinyl chloride (e.g., in sheets or microtiter wells), polystyrene latex (e.g., in beads or microtiter plates, polyvinylidene fluoride (known as Immunolon™), diazotized paper, nylon membranes, activated beads, and Protein A beads. For example, Dynatech Immunolon™ 1 or Immunolon™ 2 microtiter plates or 0.25-inch polystyrene beads (Precision Plastic Ball) can be used in the heterogeneous format. The solid support containing the antigenic peptides is typically washed after separating it from the biological sample, and prior to detection of the bound antibodies. Both standard and competitive formats are known in the art.

In a homogeneous format, the test sample is incubated with the combination of antigens in solution. For example, it may be under conditions that will precipitate any antigen-antibody that is formed. Both standard and competitive formats for these assays are known in the art.

The present invention also relates to a diagnostic kit for use in a method as described above, said kit comprising at least one polypeptide according to the invention, with said polypeptide possibly bound to a solid support. In a preferred embodiment said kit may comprise a range of said polypeptides, wherein said polypeptides are attached to specific locations on a solid substrate. More preferably, said solid support is a membrane strip and said polypeptides are coupled to the membrane in the form of parallel lines.

The present invention further relates to the polypeptides of the invention for use as an active substance in a vaccine or a medicament. Alternatively, anti-idiotypic antibodies that specifically bind an antibody of the present invention can be used as immunogens.

The present invention further relates to the use of a polypeptide or an anti-idiotypic antibody as described above for the preparation of a vaccine for the prevention or treatment of HBV infections.

The present invention also relates to a vaccine composition comprising as an active substance a polypeptide or an anti-idiotypic antibody of the invention that can be used as an inoculum to vaccinate humans (or non human mammals) against infection with HBV or to therapeutically vaccinate human (or non human mammal) carriers of HBV.

- 5 The terms "vaccine" or "vaccine composition" relate to an immunogenic composition capable of eliciting protection against HBV, whether partial or complete. The term "as an active substance" relates to the component of the vaccine composition that elicits protection against HBV. An active substance (i.e. the polypeptides of the present invention) can be used as such, in a biotinylated form (as explained in WO 93/18054)
- 10 and/or complexed to *Neutralite Avidin* according to the manufacturer's instruction sheet (Molecular Probes Inc., Eugene, OR). It should also be noted that a "vaccine" or a "vaccine composition" comprises, in addition to an active substance, a suitable excipient, diluent, carrier and/or adjuvant that, by them, do not induce the production of antibodies harmful to the individual receiving the composition nor do they elicit
- 15 protection. Suitable carriers are typically large slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers and inactive virus particles. Such carriers are well known to those skilled in the art. Preferred adjuvants to enhance effectiveness of the composition include, but are not limited to: aluminium hydroxide, aluminium in
- 20 combination with 3-O-deacylated monophosphoryl lipid A as described in WO 93/19780, aluminium phosphate as described in WO 93/24148, N-acetyl-muramyl-L-threonyl-D-isoglutamine as described in U.S. Patent N° 4,606,918, N-acetyl-normuramyl-L-alanyl-D-isoglutamine, N-acetylmuramyl-L-alanyl-D-isoglutamyl-L-alanine2(1'2'dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy) ethylamine and RIBI
- 25 (ImmunoChem Research Inc., Hamilton, MT) which contains monophosphoryl lipid A, detoxified endotoxin, trehalose-6,6-dimycolate, and cell wall skeleton (MPL + TDM + CWS) in a 2% squalene/Tween 80 emulsion. Any of the three components MPL, TDM or CWS may also be used alone or combined 2 by 2. Additionally, adjuvants such as Stimulon (Cambridge Bioscience, Worcester, MA) or SAF-1
- 30 (Syntex), RC259 (Coryxa), SBAS2, SBAS3, SBAS4, SBAS5, SBAS6, SBAS7, SBAS8 (Smith Kline Beecham, Rixensart, Belgium) may be used. Furthermore, Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA) may be used for non-human applications and research purposes. A "vaccine composition" will

further contain excipients and diluents, which are inherently non-toxic and non-therapeutic, such as water, saline, glycerol, ethanol, wetting or emulsifying agents, pH buffering substances, preservatives, and the like. Typically, a vaccine composition is prepared as an injectable, either as a liquid solution or suspension. Solid forms, suitable for solution on, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation may also be emulsified or encapsulated in liposomes for enhancing adjuvant effect. The polypeptides may also be incorporated into Immune Stimulating Complexes together with saponins, for example Quil A (ISCOMS). Vaccine compositions comprise an immunologically effective amount of the polypeptides of the present invention, as well as any other of the above-mentioned components. "Immunologically effective amount" means that the administration of that amount to an individual, either in a single dosis or as part of a series, is effective for prevention or treatment. This amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of the individual to be treated (e.g. nonhuman primate, primate, etc.), the capacity of the individual's immune system to mount an effective immune response, the degree of protection desired, the formulation of the vaccine, the treating's doctor assessment, the strain of the infecting HBV, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials. Usually, the amount will vary from 0.01 to 1000 $\mu\text{g}/\text{dose}$, more particularly from 0.1 to 100 $\mu\text{g}/\text{dose}$. The vaccine compositions are conventionally administered parenterally, typically by injection, for example, subcutaneously or intramuscularly. Additional formulations suitable for other methods of administration include oral formulations and suppositories. Dosage treatment may be a single dose schedule or a multiple dose schedule. The vaccine may be administered in conjunction with other immunoregulatory agents. It should be noted that a vaccine might also be useful for treatment of an individual, in which case it is called a "therapeutic vaccine".

The present invention also relates to a HBV genotype G specific ligand or to a HBV genotype G specific antibody specifically raised upon immunization with at least one HBV genotype G specific polypeptide of the invention, with said antibody being specifically reactive with said polypeptide and not reactive with a peptide characteristic for HBV genotype A, B, C, D, E and/or F. In a preferred embodiment,

the antibody specifically recognizes the HBV polymerase, X protein, preCore, HBcAg, HBeAg, preS1, preS2 or HBsAg of HBV genotype G.

The present invention also relates to a universal ligand or to a universal antibody specifically raised upon immunization with a universal polypeptide of the invention, with said antibody being reactive with said universal polypeptide. In a preferred embodiment, the antibody specifically recognizes the HBV polymerase, preCore, HBcAg, HBeAg, preS1, preS2 or HBsAg protein of genotypes A, B, C, D, E, F and G.

The antibody may be polyclonal or monoclonal. Also fragments derived from these monoclonal antibodies such as Fab, F(ab)₂, scFv ("single chain variable fragment") and other antibody-like constructs that retain the variable region of the antibody, providing they have retained the original binding properties, fall within the scope of the present invention and can be used in a method of the present invention. Such fragments are commonly generated by, for instance, enzymatic digestion of the antibodies with papain, pepsin, or other proteases. It is well known to the person skilled in the art that monoclonal antibodies, or fragments thereof, can be modified for various uses. Also mini-antibodies and multivalent antibodies such as diabodies, triabodies, tetravalent antibodies and peptabodies can be used in a method of the invention. The preparation and use of these fragments and multivalent antibodies has been described extensively in International Patent Application WO 98/29442. The monoclonal antibodies used in a method of the invention may be humanized versions of the mouse monoclonal antibodies made by means of recombinant DNA technology, departing from the mouse and/or human genomic DNA sequences coding for H and L chains or from cDNA clones coding for H and L chains. Alternatively the monoclonal antibodies used in a method of the invention may be human monoclonal antibodies. The term "humanized antibody" means that at least a portion of the framework regions of an immunoglobulin is derived from human immunoglobulin sequences. The antibodies used in a method of the present invention may be labeled by an appropriate label of the enzymatic, fluorescent, or radioactive type.

The present invention further relates to the use of a HBV genotype G specific antibody or ligand of the invention in a method for detecting a HBV genotype G specific polypeptide of the invention, preferably for detecting the HBcAg and/or

HBeAg of the heretofore unidentified HBV genotype G, present in a biological sample.

The present invention also relates to a method for detecting a HBV genotype G specific polypeptide of the invention, preferably for detecting the HBcAg and/or

5 HBeAg of the heretofore unidentified HBV genotype G present in a biological sample, comprising the following steps:

- (i) bringing said biological sample into contact with a HBV genotype G specific antibody or ligand of the invention recognizing said HBV genotype G specific polypeptide, under conditions being suitable for
10 producing an antigen-antibody or an antigen-ligand complex; and
- (ii) detecting the immunological binding of said antibody or ligand to said polypeptide.

The present invention further relates to the use of a universal antibody or ligand of the invention in a method for detecting a universal polypeptide of the invention, present
15 in a biological sample.

The present invention also relates to a method for detecting a universal polypeptide of the invention, present in a biological sample, comprising the following steps:

- (i) bringing said biological sample into contact with a universal antibody or ligand of the invention recognizing said universal polypeptide, under
20 conditions being suitable for producing an antigen-antibody or an antigen-ligand complex; and
- (ii) detecting the immunological binding of said antibody or ligand to said polypeptide.

The process for the detection of the immunological binding can then be carried out by
25 bringing together said polypeptide-antibody complex formed by the polypeptide of the invention and the antibody recognizing said polypeptide with:

- (i) a secondary antibody (or detector antibody) recognizing a specific epitope of the polypeptide-antibody complex but not recognizing the primary antibody alone;
- (ii) a marker either for specific tagging or coupling with said secondary
30 antibody, with said marker being any possible marker known to the person skilled in the art;

(iii) appropriate buffer solutions for carrying out the immunological reaction between the secondary antibody and the polypeptide-primary antibody complex and/or the bound secondary antibody and the marker on the other hand.

5 Advantageously, the antibodies used in the invention are in an immobilized state on a suitable support. The secondary antibody itself may carry a marker or a group for direct or indirect coupling with a marker. Alternatively, the present process may be put into practice by using any other immunoassay format known to the person skilled in the art.

10 The term "epitope" refers to that portion of the polypeptide-antibody complex that is specifically bound by an antibody-combining site. Epitopes may be determined by any of the techniques known in the art or may be predicted by a variety of computer prediction models known in the art.

The expression "recognizing", "reacting with", "immunological binding" or
15 "producing a polypeptide-antibody complex" as used in the present invention is to be interpreted that binding between the polypeptide and the antibody occurs under all conditions that respect the immunological properties of the antibody and the polypeptide of the invention.

The terms "specifically recognizing", "specifically binding to", "specifically reactive
20 with" or the like as used in the present invention in respect of antibodies and ligands are to be interpreted that the antibody or the ligand binds to a polypeptide that is unique to the heretofore unidentified HBV genotype G (and not to any polypeptide of the HBV genotypes A to F) or that the antibody or the ligand binds to a polypeptide that is universal to HBV genotypes A, B, C, D, E, F and G but not to any other
25 polypeptide.

The present invention also relates to a diagnostic kit for use in the detection of a heretofore unidentified HBV genotype G present in a biological sample, said kit comprising at least one HBV genotype G specific antibody according to the invention. In a specific embodiment the present invention relates to a diagnostic kit for use in the
30 detection of a heretofore unidentified HBV genotype G present in a biological sample, said kit comprising:

- (i) at least a primary antibody recognizing a HBV genotype G specific polypeptide of the invention (or capturing antibody);

- (ii) possibly, a secondary antibody (or detector antibody) forming an immunological complex with an epitope of the polypeptide-primary antibody complex but not with the primary antibody alone;
- (iii) possibly, a marker either for specific tagging or coupling with said secondary antibody;
- (iv) possibly, appropriate buffer solutions for carrying out the immunological reaction between the primary antibody and the polypeptide of the invention, between the secondary antibody and the polypeptide-primary antibody complex and/or between the bound secondary antibody and the marker;
- (v) possibly, for standardization purposes, a purified or synthetic peptide that is specifically recognized by the antibodies of the kit.

The present invention also relates to a diagnostic kit for use in the universal detection of HBV present in a biological sample, said kit comprising at least one universal antibody according to the invention.

In a specific embodiment the present invention relates to a diagnostic kit for use in the universal detection of HBV present in a biological sample, said kit comprising:

- (i) at least a primary antibody recognizing a universal polypeptide of the invention (or capturing antibody);
- (ii) possibly, a secondary antibody (or detector antibody) forming an immunological complex with an epitope of the polypeptide-primary antibody complex but not with the primary antibody alone;
- (iii) possibly, a marker either for specific tagging or coupling with said secondary antibody;
- (iv) possibly, appropriate buffer solutions for carrying out the immunological reaction between the primary antibody and the polypeptide of the invention, between the secondary antibody and the polypeptide-primary antibody complex and/or between the bound secondary antibody and the marker;
- (v) possibly, for standardization purposes, a purified or synthetic peptide that is specifically recognized by the antibodies of the kit.

The present invention further relates to polypeptide, an antibody or a ligand of the invention for use as a medicament.

The present invention further relates to the use of a peptide, an antibody or a ligand of the present invention for the preparation of a medicament to prevent humans of HBV infection or to treat humans with HBV infection.

The present invention also relates to a pharmaceutical composition or medicament comprising as an active substance a peptide, an antibody or a ligand of the present invention that can be used for the treatment of HBV infection.

The term "medicament" or "pharmaceutical composition" (both terms can be used interchangeable) refers to a composition comprising an antibody according to the present invention, possibly in the presence of suitable excipients known to the skilled

man such as saline, Ringer's solution, dextrose solution, Hank's solution, fixed oils, ethyl oleate, 5% dextrose in saline, substances that enhance isotonicity and chemical stability, buffers and preservatives. The "medicament" may be administered by any suitable method within the knowledge of the skilled man. The preferred route of administration is parenterally. In parental administration, the medicament of this invention will be formulated in a unit dosage injectable form such as a solution, suspension or emulsion, in association with the pharmaceutically acceptable excipients as defined above. However, the dosage and mode of administration will depend on the individual. Generally, the medicament is administered so that the antibody is given at a dose between 1 $\mu\text{g/kg}$ and 10 mg/kg , more preferably between 10 $\mu\text{g/kg}$ and 5 mg/kg , most preferably between 0.1 and 2 mg/kg . Preferably, it is given as a bolus dose. Continuous infusion may also be used. If so, the medicament may be infused at a dose between 5 and 20 $\mu\text{g/kg/minute}$, more preferably between 7 and 15 $\mu\text{g/kg/minute}$. Dosage forms suitable for oral administration include but are not limited to tablets, pills, capsules, caplets, granules, powders, elixirs, syrups, solutions and aqueous or oil suspensions. A suitable daily dose of the active compound for administration to human beings is generally from about 1 mg to about 5000 mg, more usually from about 5 mg to about 1000 mg, given in a single dose or in divided doses at one or more times during the day.

It should also be clear that the pharmaceutical composition of the present invention might comprise a functionally equivalent variant of the antibody of the invention. The latter terms refer to a molecule which contains the antibody of the invention, to which certain modifications have been applied, and which retains all or part of its biological properties. Such modifications include but are not limited to the addition of

polysaccharide chains, the addition of certain chemical groups, the addition of lipid moieties, the fusion with other peptide or protein sequences and the formation of intramolecular cross-links.

The present invention also provides methods for identifying compounds or agents
5 (herein referred to as "test compound") that can be used to treat disorders characterized by (or associated with) HBV infection. These methods are also referred to herein as "drug screening assays" or "bioassays."

In one embodiment, the drug screening assay may include the step of screening a test compound for the ability to interact with (e.g., bind to) an HBV genotype G protein,
10 or any functionally equivalent part thereof, to modulate the interaction of an HBV protein and a target molecule. Typically, the assay is a cell-free assay including the following steps:

- (i) combining the HBV genotype G protein of the invention or an immunogenic fragments thereof, and a test compound under conditions
15 which allow for interaction of (e.g., binding of) the test compound to the HBV genotype G protein or a portion thereof to form a complex;
- (ii) detecting the formation of a complex.

The ability of the test compound to interact with (e.g. bind to) the HBV genotype G protein or portion thereof is indicated by the presence of the test compound in the
20 complex. Formation of complexes between the HBV genotype G protein and the test compound can be quantitated, for example, using a standard immunoassay. The HBV genotype G protein or its immunogenic fragment employed in such a test, may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly.

25 In another embodiment, the invention provides a screening assay to identify test compounds which modulate (e.g., stimulate or inhibit) the interaction (and most likely HBV genotype G protein activity as well) between an HBV genotype G protein and:

- a molecule (target molecule) to which the HBV genotype G protein normally interacts; or
- 30 - antibodies which specifically recognize the HBV genotype G protein.

Target molecules include proteins in the same signaling path as the HBV genotype G protein, e.g., proteins which may function upstream (including both stimulators and inhibitors of activity) or downstream of the HBV protein signaling pathway [Zn-

fingers, protease activity, regulators of cysteine redox status]. Typically, the assay is a cell-free assays, which includes the following steps:

- (i) combining an HBV genotype G protein or a immunogenic fragment thereof, an HBV protein target molecule (e.g., an HBV protein ligand) or a specific antibody and a test compound under conditions which allow for interaction of (e.g., binding of) the HBV genotype G protein or a portion thereof with the target molecule or the antibody;
- (ii) detecting the formation of a complex which includes the HBV genotype G protein and the target molecule or the antibody, or detecting the interaction/reaction of the HBV genotype G protein and the target molecule or antibody.

To perform the above-described drug-screening assay, it is feasible to immobilize either the HBV genotype G protein or its target molecule to facilitate separation and to accommodate automation of the assay. Detection of complex formation can include direct quantitation of the complex by, for example, measuring inductive effects of the HBV genotype G protein. A statistically significant change, such as a decrease, in the interaction of the HBV genotype G protein and the target molecule (e.g., in the formation of a complex between the HBV genotype G protein and the target molecule) in the presence of a test compound (relative to what is detected in the absence of the test compound) is indicative of a modulation (e.g., stimulation or inhibition) of the interaction between the HBV genotype G protein and the target molecule. Modulation of the formation of complexes between the HBV genotype G protein and the target molecule can be quantitated using, for example, an immunoassay.

It should be clear that a modulator of the interaction between a HBV genotype G protein and a target molecule, when identified by any of the herein-described methods, is contemplated in the invention.

Another technique for drug screening which provides for high throughput screening of compounds having suitable binding affinity to the HBV genotype G protein is described in detail in "Determination of Amino Acid Sequence Antigenicity" by Geysen HN, WO Application 84/03564, published on 13/09/84, and incorporated herein by reference. In summary, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other

surface. The protein test compounds are reacted with fragments of HBV genotype G protein and washed. Bound HBV genotype G protein is then detected by methods well known in the art.

5 This invention also contemplates the use of a competitive drug screening assays in which neutralizing antibodies capable of binding a HBV genotype G protein specifically compete with a test compound for binding the HBV genotype G protein. In this manner, the antibodies can be used to detect the presence of any protein that shares one or more antigenic determinants with the HBV genotype G protein.

10 In yet another embodiment, the invention provides a method for identifying a compound capable of modulating HBV nucleic acid expression and/or HBV protein activity. Methods for assaying the ability of a test compound to modulate the expression of a HBV nucleic acid or activity of a HBV genotype G protein are typically cell-based assays. However, HBV genotype G infected animals are also contemplated herein. HBV genotype G infected or transfected cells which transduce
15 signals via a pathway involving a HBV genotype G protein can be induced to overexpress an HBV protein in the presence and absence of a test compound. These HBV genotype G infected cells also form part of the present invention. Said HBV genotype G infected cells and animals are further also referred to as HBV genotype G infected clones.

20

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of stated integers or steps but not to the exclusion of any other integer or step or
25 group of integers or steps. The disclosures of the various patent applications, patents and/or publications that are cited, as well as the references cited in these publications, are incorporated by reference herein. This, however, does not imply that the content of all of these disclosures is to be seen as part of common general knowledge.

30 The present invention claims priority under 35 USC 119 from the US patent application No. 60/169,287, which was filed on 7 December 1999. The present invention further claims priority under PCT Article 8 from US patent application No.

60/169,287, which was filed on 7 December 1999 and from EP 99870252.6, which was filed on 3 December 1999.

EXAMPLES

Example 1: Prevalence of the different HBV genotypes

5 1.1 Sample collection

A total of 121 HBV-positive plasma samples were collected in France (n=39) and the United States (n=82). The samples were divided into aliquots and stored at -20°C until use. Samples were taken from chronic HBV carriers and were randomly selected as
10 they became available.

1.2 HBV DNA extraction and amplification

HBV DNA was extracted from 100 µl serum sample using the High Pure PCR
15 Template Preparation kit (Roche Diagnostics, Brussels, Belgium) essentially as previously described (Stuyver et al., 1999). A PCR fragment was generated covering the preS1, preS2 and HBsAg region by use of primers HBPr1 and HBPr135 (outer PCR) followed by a nested reaction using HBPr2 and HBPr94 (Fig. 4; Table 1). Outer
20 PCR amplified the viral DNA over 40 cycles, with denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and elongation at 72 °C for 30 s. Samples negative in first-round PCR were further amplified with nested PCR primers for 35 cycles with the same thermal profile.

1.3 HBV genotyping

25 The 121 serum samples were further genotyped by using a research version of the HBV genotyping Line Probe Assay (LiPA) (Van Geyt et al., 1998). The results are summarized in Table 2. A very typical but previously unrecognized reactivity pattern was obtained for 2 samples from Europe and 11 samples from the United States:
30 hybridization reactions were observed on probe 140 (specifically designed for genotype A), probe 148 (designed for genotypes A and B), probe 80 (designed for genotypes C, D and E) and probe 239 (designed for genotypes B and E) (Van Geyt et

al. 1998). Because this mixed hybridization pattern did not allow unique type recognition, further characterization was done via sequence analysis.

Example 2: Characterization of the samples that showed a mixed hybridization pattern

2.1 HBV DNA extraction and amplification

HBV DNA was extracted from 100 µl serum sample using the High Pure PCR Template Preparation kit (Roche Diagnostics, Brussels, Belgium) essentially as previously described (Stuyver et al., 1999). The complete genome of HBV was amplified using the Expand High Fidelity PCR system (Roche Diagnostics, Brussels, Belgium). The amplification was performed on 5 µl of the extracted DNA with the primers HBPr108 and HBPr109 (Table 1). A 45 µl reaction mix was made, containing 5 µl 10 x Expand High Fidelity PCR system buffer, 2.6 U Expand High Fidelity PCR system enzyme mix, 200 µM dNTPs, 300 nM of each primer and sterile H₂O. Amplification was performed with denaturation at 94 °C for 40 s, annealing (after shifting to 60 °C in 50 s) for 1 min and elongation (after shifting to 72 °C in 15 s) for 4 min, with an increment of 5 s/cycle (Günther et al., 1998). Two shorter PCR fragments were also generated: (i) the first amplicon covered the preS1, preS2, and HBsAg region and was amplified using primers HBPr1 and HBPr135 (outer PCR) followed by a nested reaction using HBPr2 and HBPr94 (Table 1); and (ii) the second amplicon covered the preCore/Core region and was amplified by means of a hemi-nested set of PCR primers (HBPr86 and HBPr303, followed by HBPr87 and HBPr303). Outer PCR amplified the viral DNA over 40 cycles, with denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s and elongation at 72 °C for 30 s. Samples negative in first-round PCR were further amplified with nested PCR primers for 35 cycles with the same thermal profile.

2.2 Sequencing

Sequencing was performed on an automated DNA sequencer ABI 377 (PE Applied Biosystems, Foster City, CA, USA), using fluorescence-labeled dideoxynucleotide

chain terminators (ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA polymerase FS; PE Applied Biosystems). The primers used for sequencing the complete genome are summarized in Table 1. The primers used to sequence the shorter PCR fragments are the same as the amplification primers.

- 5 One European virus isolate (FR1) was selected for whole genome sequencing (Fig. 1; SEQ ID NO 1), whereas the preCore/Core and preS/S genes were sequenced from another seven samples (Figs. 7 and 8; SEQ ID NO 43-56).

2.3 Homology percentages with other genotypes

10

In order to compare the genetic relatedness of the FR1 strain with 36 other complete HBV genomes, homology percentages were calculated. The FR1 sequence showed an average homology of: 87.1% (min. 86.2%, max. 87.7%) with genotype A (five sequences); 86.6% (min. 86.5%, max. 86.6%) with genotype B (four sequences);
15 86.5% (min. 86.0%, max. 87.0%) with genotype C (14 sequences); 86.9% (min. 86.3%, max. 87.3%) with genotype D (eight sequences); 88.3% (min. 88.2%, max. 88.4%) with genotype E (two sequences); and 84.7% (min. 84.5%, max. 84.8%) with genotype F (three sequences).

20 2.4 Phylogenetic distance with other genotypes

In order to compare the genetic relatedness of the FR1 strain with 36 other complete HBV genomes, also the phylogenetic distances were calculated. Phylogenetic distances between the six recognized HBV genotypes (36 sequences) were compared
25 to each other and to the FR1 strain (Fig. 9). A clear difference emerged between the phylogenetic distances (i) within one genotype (distance range of 0.01 - 0.06) and (ii) between different genotypes (distance range 0.08 - 0.17). Using a *t*-test for the mean and a distance of 0.08 as border value between genotypes, FR1 was found to be significantly different from genotypes A - F (range 0.11 - 0.17; $P < 0.019$).

30 Phylogenetic trees were created and analyzed by distance matrix comparison using DNADIST, Neighbor and Drawgram software programs of PHYLIP version 3.5c (Felsenstein, 1993). Statistical analysis (*t*-test for the mean) was performed using MedCalc Version 4.05-Windows 95 (Medcalc Software, Mariakerke, Belgium). A *P*

value of < 0.05 was considered to be statistically significant. Complete genome sequences representing the different genotypes were retrieved from the GenBank; their accession numbers are indicated in Figure 10. Phylogenetic trees of the complete genome sequences (Fig. 10a), as well as of the individual ORF [Fig. 10b for the surface region (preS1, preS2, HBsAg); data not shown for the other ORFs] were constructed, illustrating that FR1 is indeed located on a separate branch. Fig. 10b further illustrates that the HBV samples from the USA and France, are closely related to each other. Based on these calculations and illustrated by means of phylogenetic trees, FR1 and the other virus strains shown belong to a new HBV genotype, called genotype G.

Example 3: Characterization of the genome structure and open reading frames in HBV strains belonging to genotype G

The complete genome structure of FR1 was similar to that described for the known HBV genotypes, but was found to be 3248 bp long (Fig. 4).

The preCore region has translational stops at codon 2 (TAA instead of CAA) and codon 28 (TAG instead of TGG) in all eight isolates sequenced. Based on the presence of this dual stop codon, the presence of HBeAg is generally not expected. Paradoxically, sample FR2 showed the presence of HBeAg in the plasma. The Core region is 585 nucleotides long and encodes a Core protein of 195 amino acids (Fig. 5). In contrast to the other genotypes, the Core region had a nucleotide insert of 36 bp, located after the fifth nucleotide following the Core translation initiation point (A at position 1901). Therefore, the insert in the core region encoding twelve additional amino acids RTTLPYGLFGLD (SEQ ID NO 155) may be responsible for a novel mechanism of expression of HBcAg and HBeAg, and core and e antigens with unique structures and immunological properties may exist. Genotype G, as well as all other genotypes except A, showed a 6 nucleotide deletion in the carboxy-terminal part of the HBcAg ORF (Figs. 4 and 5).

The preS1 region contains 354 bp (118 amino acids), the preS2 region 165 bp (55 amino acids), and the HBsAg region 678 bp (226 amino acids) (Fig. 6). Like genotype E, genotype G strains showed a 3 nucleotide deletion (1 amino acid at position 11; Fig. 6) in the amino-terminal part of preS1. Based on the presence of a lysine (K) at

HBsAg position 122, a lysine (K) at position 160 and a proline (P) at position 127, the serological subtype of this genotype G strain was predicted to be adw2.

The polymerase region of this genotype contains 2526 bp (842 amino acids). The deletion in the carboxy-terminal part of HBcAg, as well as the deletion at the amino terminus of preS1 affects the numbering of the polymerase protein. The methionine residue, which is prone to changes during lamivudine therapy, is located at position 549 in the highly conserved YMDD motif (Bartholomeusz et al., 1998). Figure 6 also shows the exact numbering for this methionine residue in the other genotypes.

10 **Example 4: Method for the detection of HBV genotype G**

4.1 HBV DNA extraction and amplification

HBV DNA was extracted from serum samples with the High Pure PCR Template Preparation Kit (Roche Diagnostics, Brussels, Belgium). A nested set of PCR primers was designed, allowing the amplification of the HBsAg region and generating a 341 bp fragment (outer sense primer HBPr 134; outer antisense primer HBPr 135; nested sense primer HBPr 75; nested antisense primer HBPr 94; table 1). All primers were tagged with a biotin group at the 5' end. Outer PCR amplified the DNA over 40 cycles (30 s at 95°C; 30 s at 45°C; 30 s at 72°C). Samples negative in first round PCR were further amplified with nested PCR primers for 35 cycles with the same thermal profile.

4.2 Design of a LiPA for HBV genotyping

A total of 20 genotype-specific probes are designed. Probes designed for the identification of genotypes A, B, C, D, E and F are described in Van Geyt et al. (1998). By way of example, probes specifically selected for the identification of HBV genotype G nucleic acid sequences recognize the regions indicated as target A or target B in Figure 1. After enzymatic addition of a poly d(T) tail, the selected oligonucleotide probes are applied as horizontal lines on nitrocellulose membranes. One line of biotinylated DNA is applied alongside as a positive control.

4.3 Testing of the LiPA with a cloned reference panel and with representative clinical samples

The assay is essentially carried out as described for the HCV line probe assay (Stuyver et al., 1996). The reactivity of the different probes on the HBV genotyping LiPA is assessed with a cloned reference panel and with clinical samples. Figure 11 shows the result for some representative samples after hybridization of their PCR product to the probes on the LiPA. PCR fragments from HBV strain FR1 hybridize with the generic probes 140, 148, 80 and 239 and with the genotype G specific probes selected to recognize target A or target B as indicated in Figure 1.

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CLAIMS

1. An isolated and/or purified HBV polynucleic acid, having a nucleotide sequence which is unique to a heretofore unidentified HBV genotype and which is phylogenetically different from HBV genotypes A, B, C, D, E and F characterized by:
 - (a) a sequence as defined in SEQ ID NO 1; or
 - (b) a sequence with at least 90%, more preferably 91%, most preferably 92% identity to the sequence as defined in SEQ ID NO 1; or
 - (c) a sequence that is degenerate as a result of the genetic code to the sequence as defined in (a) and (b).
2. An HBV polynucleic acid according to claim 1, further characterized that its nucleotide sequence differs from HBV genotype A, B, C, D, E or F in at least one of the following characteristics:
 - (a) it has a length of 3248 basepairs;
 - (b) it encodes a translational stop codon at amino acids 2 and 28 of the preCore region;
 - (c) it has a 36 nucleotide insert in the N-terminal part of the Core region;
 - (d) it has a 6 nucleotide deletion in the C-terminal part of the HBcAg ORF;
 - (e) it has a 3 nucleotide deletion in the N-terminal part of the preS1 ORF.
3. An HBV polynucleic acid according to any of claims 1 or 2 comprising at least one of the following sequences: SEQ ID NO 44, SEQ ID NO 45, SEQ ID NO 46, SEQ ID NO 47, SEQ ID NO 48, SEQ ID NO 49, SEQ ID NO 50, SEQ ID NO 51, SEQ ID NO 53, SEQ ID NO 54, SEQ ID NO 55, SEQ ID NO 56, SEQ ID NO 57, SEQ ID NO 58, SEQ ID NO 59, SEQ ID NO 60.
4. A fragment of a polynucleic acid according to any of claims 1 to 3 or the complement thereof characterized:
 - that said fragment is unique to the heretofore unidentified HBV genotype G, containing at least one nucleotide or a combination of nucleotides

different from known HBV genotypes A, B, C, D, E or F nucleotide sequences; or

- that said fragment is a universal fragment, comprised within the nucleic acid sequence of genotypes A, B, C, D, E, F and G;

5 provided that said fragment is not equal to the preC/C gene sequences of SEQ ID NO 7 or SEQ ID NO 169 or to the preS/S sequence of SEQ ID NO 8.

5. A method for the detection in a biological sample of the presence of a polynucleic acid according to any of claims 1 to 4.

10

6. A method according to claim 5 further characterized that it comprises the following steps:

- (i) possibly extracting the nucleic acid present in the biological sample;
- (ii) amplifying the nucleic acid;
- 15 (iii) detecting the amplified nucleic acid.

7. A method according to any of claims 5 to 6 further characterized that it comprises the following steps:

20

- (i) possibly extracting the nucleic acid present in the biological sample;
- (ii) amplifying the nucleic acid;
- (iii) hybridizing the nucleic acid, possibly under denatured conditions, at appropriate conditions with one or more probes, with said probes being possibly attached to a solid substrate;
- (iv) possibly washing at appropriate conditions;
- 25 (v) detecting the hybrids formed.

8. A method according to any of claims 5 to 6 further characterized that it comprises the following steps:

30

- (i) possibly extracting the nucleic acid present in the biological sample;
- (ii) amplifying the nucleic acid;
- (iii) sequencing the nucleic acid.

9. An oligonucleotide primer comprising a fragment according to claim 4, with said primer being able to act as primer for specifically sequencing or specifically amplifying a nucleic acid or part of a nucleic acid according to any of claims 1 to 3.
- 5
10. An oligonucleotide probe comprising a fragment according to claim 4, with said probe being able to act as a hybridization probe for specific detection of a nucleic acid or part of a nucleic acid according to any of claims 1 to 3.
- 10
11. An oligonucleotide primer or an oligonucleotide probe according to any of claims 9 to 10 further characterized that it recognizes one of the following target sequences: SEQ ID NO 62 to SEQ ID NO 154.
12. A method for HBV genotyping according to any of claims 5 to 8, making use of at least one primer according to claim 9 or claim 11 and/or at least one probe according to claim 10 or claim 11.
- 15
13. A diagnostic kit for use in a method according to any of claims 5 to 8 and/or 12, said kit comprising at least one primer according to claim 9 or claim 11 and/or at least one probe according to claim 10 or claim 11.
- 20
14. A diagnostic kit for use in a method according to any of claims 5 to 7 and/or 12, said kit comprising the following components:
- (i) when appropriate, a means for releasing, isolating or concentrating the nucleic acids present in said sample;
 - 25 (ii) a primer pair or a primer mix;
 - (iii) at least one probe according to any of claims 10 to 11, with said probe specifically hybridizing with a nucleic acid characteristic to HBV genotype G, or a universal probe, fixed to a solid support;
 - 30 (iv) a hybridization buffer, or components necessary for producing said buffer;
 - (v) a wash solution, or components necessary for producing said solution;

(vi) when appropriate, a means for detecting the hybrids resulting from the preceding hybridization.

5 15. A polypeptide having an amino acid sequence encoded by a polynucleic acid according to any of claims 1 to 4.

10 16. A polypeptide according to claim 15, comprising at least one of the following sequences: SEQ ID NO 2, SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6.

17. A polypeptide according to claim 15, comprising at least one of the following sequences: SEQ ID NO 155 to SEQ ID NO168.

15 18. A polypeptide according to any of claims 14 to 17 which is recombinantly expressed.

20 19. An expression vector comprising a polynucleic acid or part thereof according to any of claims 1 to 4 operably linked to prokaryotic, eukaryotic or viral transcription and translation control elements.

20. A host cell transformed with an expression vector according to claim 19.

21. A method for the production of a recombinant polypeptide according to claim 18 comprising the following steps:

- 25 (i) transformation of an appropriate cellular host with an expression vector according to claim 19;
- (ii) culturing said transformed cellular host under conditions enabling the expression of the polynucleic acid inserted in the expression vector; and
- (iii) harvesting said polypeptide.

30 22. A method for detecting antibodies present in a biological sample, comprising the following steps:

- (i) contacting the biological sample with a polypeptide according to any of claims 15 to 18;
- (ii) detecting the immunological complex formed between said antibodies and said polypeptide.

5

23. A method for HBV genotyping of a biological sample, comprising the following steps:

- (i) contacting the biological sample with at least one polypeptide according to any of claims 15 to 18;
- 10 (ii) detecting the immunological complex formed between the antibodies present in said biological sample and said polypeptide.

24. A diagnostic kit for use in a method according to any of claims 22 to 23, said kit comprising at least one polypeptide according to any of claims 15 to 18, with said
15 polypeptide possibly bound to a solid support.

25. A ligand specifically binding a polypeptide according to any of claims 15 to 18.

26. An antibody raised upon immunization with at least one polypeptide according to
20 any of claims 15 or 18, with said antibody being specifically reactive with any of said polypeptides.

27. A method for detecting a polypeptide according to any of claims 15 to 18 comprising the following steps:

- 25 (i) contacting said polypeptide with a ligand according to claim 25, binding specifically to said polypeptide;
- (ii) determining the immunological complex formed between said polypeptide and said ligand.

30 28. A method for detecting a polypeptide according to any of claims 15 to 18 comprising the following steps:

- (i) contacting said polypeptide with an antibody according to claim 26, specifically reactive with said polypeptide;

- (ii) determining the immunological complex formed between said polypeptide and said antibody.

29. A method for detecting a HBcAg or a HBeAg specific to a heretofore
5 unidentified HBV genotype of claim 1, comprising the following steps:

- (i) contacting said polypeptide with a ligand according to claim 25, specifically binding to said polypeptide;
- (ii) determining the immunological complex formed between said polypeptide and said ligand.

10

30. A method for detecting a HBcAg or a HBeAg specific to a heretofore
unidentified HBV genotype of claim 1, comprising the following steps:

- (i) contacting said polypeptide with an antibody according to claim 26, specifically reactive with said polypeptide;
- (ii) determining the immunological complex formed between said polypeptide and said antibody.

15

31. A diagnostic kit for use in the detection of a heretofore unidentified HBV
genotype present in a biological sample, said kit comprising at least one ligand
20 according to claim 25 or at least one antibody according to claim 26.

20

32. A polypeptide according to any of claims 15 to 18, a ligand according to claim 25
and/or an antibody according to claim 26 for use as active substances in a vaccine
or a medicament.

25

33. Use of a polypeptide according to any of claims 15 to 18, a ligand according to
claim 25 and/or an antibody according to claim 26 for the preparation of a
vaccine or a medicament for the prevention or treatment of HBV infections.

30 34. A pharmaceutical composition comprising at least a polypeptide according to any
of claims 15 to 18, a ligand according to claim 25 and/or an antibody according to
claim 26 and a pharmaceutically acceptable carrier.

35. A pharmaceutical composition according to claim 34 for use in a method to prevent or treat HBV infections.
36. A vaccine for immunizing a mammal against HBV infection, comprising at least
5 a polypeptide according to any of claims 15 to 18 in a pharmaceutically acceptable carrier.
37. The use of a polypeptide of any of claims 15 to 18 in a drug-screening assay.
- 10 38. The use of a HBV genotype G infected clone in a drug-screening assay.

Figure 1

	1850	
preC	ATGTAACCTTTTACACCTCTGCGCTAATCATCTCTTGTTCATGTCCTACTGTTCAGCCTTCC	
HBX	M - L F H L C L I I S C S C P T V Q A S	
	C N F F T S A - (SEQ ID NO 2)	
	1900	
preC/C	AAGCTGTGCGCTTGGGTGGCTTTAGGGCATGGATAGAACAACTTTGCCATATGGCCTTTT	
	K L C L G W L - G M D R T T L P Y G L F	
	(SEQ ID NO 3)	
	1950	
Core	GGCTTAGACATTGACCCCTTATAAAGAATTGGAGCTACTGTGGAGTTGCTCTCGTTTGTG	
	G L D I D P Y K E F G A T V E L L S F L	
	2000	
Core	CCTTCGTGACTTTTTCGCCGTCTGTTCGTGATCTTCTCGACACCCGCTTCAGCTTTGTACCGG	
	P S D F F P S V R D L L D T A S A L Y R	
	2050	
	2100	
Core	GAATCCTTAGAGTCCTCTGATCATTTGTTGCGCTCACCATACAGCACTCAGGCAAGCAATC	
	E S L E S S D H C S P H H T A L R Q A I	

Figure 1 - cont'd 1

	2150	
	:	:
	: CTGTCTGGGGTGAGTTGATGACTCTAGCTACCTGGGTGGGTAATAATTGGAAGATCCA	:
Core	L C W G E L M T L A T W V G N N L E D P	
	2200	
	:	:
	: GCATCCAGAGATTGGTGGTCAATTATGTTAATACTAATATGGGTTTAAAAATCAGGCAA	:
Core	A S R D L V V N Y V N T N M G L K I R Q	
	2250	
	:	:
	: CTATTGTGGTTTCACATTTCCTGTCTTACTTTTGGGAGAGAAACCGTTCTTGAGTATTG	:
Core	L L W F H I S C L T F G R E T V L E Y L	
	2300	2350
	:	:
	: GTGTCTTTTGGAGTGTGGATTGCGCACTCCTCCTGCTTATAGACCACCAATGCCCTATC	:
Core	V S F G V W I R T P P A Y R P P N A P I	
HBP01		M P L S

Figure 1 - cont'd 2

		2400
	: : : : : :	
Core	CTATCAACACTTCGGAGACTACTGTTGTAGACGAAGGCGAGTCCCCTCGAAGAAGA	
	L S T L P E T T V V R R R G R S P R R R	
HBPOL	Y Q H F R R L L L L D E E A G P L E E E	
		2450
	: : : : : :	
Core	ACTCCCTCGCCTCGCAGACGAAGATCTCAATCGCCGCGTCGCAGAAGATCTGCATCTCCA	
	T P S P R R R R S Q S P R R R R S A S P	
HBPOL	L P R L A D E D L N R R V A E D L H L Q	
		2500
	: : : : : :	
Core	GCTTCCCAATGTTAGTATTCTTGGACTCACAAGGTGGGAACTTTACGGGGCTGTATTC	
	A S Q C - (SEQ ID NO 4)	
HBPOL	L P N V S I P W T H K V G N F T G L Y S	
		2550
	: : : : : :	
HBPOL	TTCTACTATACCTGTCTTTAATCCTGATTGGCAAACTCCTTCTTTTCCAAATATCCATT	
	S T I P V F N P D W Q T P S F P N I H L	

Figure 1 - cont'd 3

2600 2650
 : : : : :
 GCATCAAGACATTATAACTAAATGTGAACAATTTGTGGGCCCTCTCACAGTAAATGAGAA
 HBPOL H Q D I I T K C E Q F V G P L T V N E K

2700
 : : : : :
 ACGAAGATTAAAACTAGTTATGCTGCTGCCAGATTTTCCCAAACCTCTACTATAATATTACC
 HBPOL R R L K L V M P A R F F P N S T K Y L P

2750
 : : : : :
 ATTAGACAAAGGTATCAAAACCGTATTATCCAGAAAATGTAGTTAATCATTACTTCCAGAC
 HBPOL L D K G I K P Y Y P E N V N H Y F Q T

2800
 : : : : :
 CAGACATTATTACATACCCCTTTGGAAGCGGGGTATTCTATATAAGAGAGAAACATCCCG
 HBPOL R H Y L H T L W K A G I L Y K R E T S R

Figure 1 - cont'd 5

	:	:	:	:	: : :	
	<u>CCGGTTCA</u>	<u>CCCCCTCCA</u>	CACGAGGCCTTTTGGGTGGAGGCCCTCAGTCATCAGGGCACAG R V H P S T R R P F G V E P S V S G H T P G F T P P H G G L L G W S P Q S Q G T			
HBP01 pres1						
	:	:	:	:	: : :	
		3150				
	:	:	:	:	: : :	
	<u>TAACTTGCCAGCAGATCGGCCTCCTGCCTCCACC</u>	AATCGTCAGTCAGGGAGGCAGC N N F A S R S A S C L H Q S S V R E A A L T T L P A D P P P A S T N R Q S G R Q				
HBP01 pres1						
	:	:	:	:	: : :	
		3200			3248 1	
	:	:	:	:	: : :	
	<u>CTACTCCC</u>	<u>ATCTCTCCACC</u>	<u>ACTAAGACAGTCATCCTCAGGCCATGCAGTGGA</u>	<u>ACTCTA</u> Y S H L S T T K R Q S S S G H A V E L Y P T P I S P P L R D S H P Q A M Q W N S		
HBP01 pres1/S2						
	:	:	:	:	: : :	
		50				
	:	:	:	:	: : :	
	<u>CAGCATTC</u>	<u>CACCAAGCTCTACA</u>	<u>ATAATCCCAAAGTCAGGGCCCTGTA</u>	<u>TTTTCTCTGCTGGTG</u> S I P P S S T K S Q S Q G P V F S C W W T A F H Q A L Q N P K V R G L Y F P A G		
HBP01 pres2						

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Figure 1 - cont'd 8

	550		600
	:	:	:
	ACTCTATGATCCCTCATGTTGCTGTACAAAACCTTCGGACGGAATTCACCTGTATTC		
HBPol	L Y V S L M L L Y K T F G R K L H L Y S		
HBS	N S M Y P S C C C T K P S D G N C T C I		
		650	
	:	:	:
	CCATCCCATCATCTTGGGCTTTCGCAAAATACCTATGGGAGTGGGCTCAGTCCGTTTCT		
HBPol	H P I I L G F R K I P M G V G L S P F L		
HBS	P I P S S W A F A K Y L W E W A S V R F		
		700	
	:	:	:
	CTTGGCTCAGTTTACTAGTGCCATTGTGTTTCAGTGGTTCGTAGGGCTTCCCCCACTGTCT		
HBPol	L A Q F T S A I C S V V R R A F P H C L		
HBS	S W L S L L V P F V Q W F V G L S P T V		
		750	
	:	:	:
	GGCTTTCAGCTATATGGATGATGTGGTATTTGGGGGCCAAATCTGTACAACATCTTGAGTC		
HBPol	A F S Y M D D V V L G A K S V Q H L E S		
HBS	W L S A I W M M W Y W G P N L Y N I L S		

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Figure 1 - cont'd 9

	800		
	:	:	:
	CCTTTATACCGCTGTACCAATTTCTTTTGTCTTTGGTATACATCTAAACCCCTAACAA		
HBPOL	L Y T A V T N F L L S L G I H L N P N K		
HBS	P F I P L L P I F F C L W V Y I - (SEQ ID NO 5)		
		850	900
	:	:	:
	AACAAAAGATGGGTTATTCCTTAAATTTTATGGGATATGTAATTGGAAGTTGGGGTAC		
HBPOL	T K R W G Y S L N F M G Y V I G S W G T		
			950
	:	:	:
	TTTGCCACAAGAACACATCACACAGAAAATTAAAGCAATGTTTTCGGAAACTCCCTGTAA		
HBPOL	L P Q E H I T Q K I K Q C F R K L P V N		
			1000
	:	:	:
	CAGGCCAATTGATTGGAAAGTCTGTCAACGAATAACTGGTCTGTTGGGTTTCGCTGCTCC		
HBPOL	R P I D W K V C Q R I T G L L G F A A P		

Figure 1 - cont'd 11

		1350	
	:	:	:
	TGGGACTGACAAATTCTGTGTCCTTTCTCGGAAATATACATCCTTTCCATGGCTGCTAGG		
HBX			M A A R
HBPol	G T D N S V V L S R K Y T S F P W L L G		
		1400	
	:	:	:
	CTGTGCTGCCAACTGGATCCTTTCGGGGACGTCCTTTGTTTACGTCCCGTCAGCGCTGAA		
HBX	L C C Q L D P S R D V L C L R P V S A E		
HBPol	C A A N W I L R G T S F V Y V P S A L N		
		1450	1500
	:	:	:
	TCCAGCGGACGACCCCTCCCGGGCCGTTTGGGGCTCTGTGCGCCCTTCTCCGTCGCGC		
HBX	S S G R P L P G P F G A L S P P S P S A		
HBPol	P A D D P S R G R L G L C R P L L R L P		
		1550	
	:	:	:
	GTTCCCTGCCGACCGGGGCGACCTCTCTTTACGGGCTCTCCCGTCTGTTCCTCTCA		
HBX	V P A D H G A H L S L R G L P V C S F S		
HBPol	F L P T T G R T S L Y A V S P S V P S H		

Figure 1 - cont'd 12

1600
 HBX TCTGCCGGACCGTGTGCACTTCGCTTCACCTCTGCACGTTACATGGAAACCGCCATGAAC
 S A G P C A L R F T S A R Y M E T A M N
 HBPOL L P D R V H F A S P L H V T W K P P - (SEQ ID NO 6)

1650
 HBX ACCTCTCATCTGCCAAGCAGTTATATAAGTGGACTCTTGGACTGTTTGTATGTCA
 T S H H L P R Q L Y K W T L G L F V M S

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1700
 HBX ACAACCGGGTGGAGAAATACTTCAAGGACTGTGTTTTTGTGCTGAGTGGGAAGAATTAGGC
 T T G V E K Y F K D C V F A E W E L G

1750 1800
 HBX AATGAGTCCAGGTTAATGACCTTTGTATTAGGAGGCTGTAGGCATAAATTGGTCTGCGCA
 N E S R L M T F V L G G C R H K L V C A

CCAGCACC (SEQ ID NO 1)
 P A
 HBX

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ATGCAACTTT TTCACCTCTG CCTAATCATC TCTTGTTTCAT
GTCCTACTGT TCAAGCCTCC AAGCTGTGCC TTGGGTGGCT
TTAGGGCATG GATAGAACAA CTTTGCCATA TGGCCTTTTT
GGCTTAGACA TTGACCCTTA TAAAGAATTT GGAGCTACTG
TGGAGTTGCT CTCGTTTTTG CCTTCTGACT TTTTCCCGTC
TGTTTCGTGAT CTTCTCGACA CCGCTTCAGC TTTGTACCGG
GAATCCTTAG AGTCCTCTGA TCATTGTTTCG CCTCACCATA
CAGCACTCAG GCAAGCAATT CTGTGCTGGG GTGAGTTGAT
GACTCTAGCT ACCTGGGTGG GTAATAATTT GGAAGATCCA
GCATCCAGAG ATTTGGTGGT CAATTATGTT AATACTAATA
TGGGTTTAAA AATCAGGCAA CTATTGTGGT TTCACATTTT
CTGTCTTACT TTTGGGAGAG AAACCGTTCT TGAGTATTTG
GTGTCTTTTG GAGTGTGGAT TCGCACTCCT CCTGCTTATA
GACCACCAA TGCCCCTATC CTATCAACAC TTCCGGAGAC
TACTGTTGTT AGACGAAGAG GCAGGTCCCC TCGAAGAAGA
ACTCCCTCGC CTCGCAGACG AAGATCTCAA TCGCCGCGTC
GCAGAAGATC TGCATCTCCA GCTTCCCAAT GTTAG
(SEQ ID NO 7)

Figure 2

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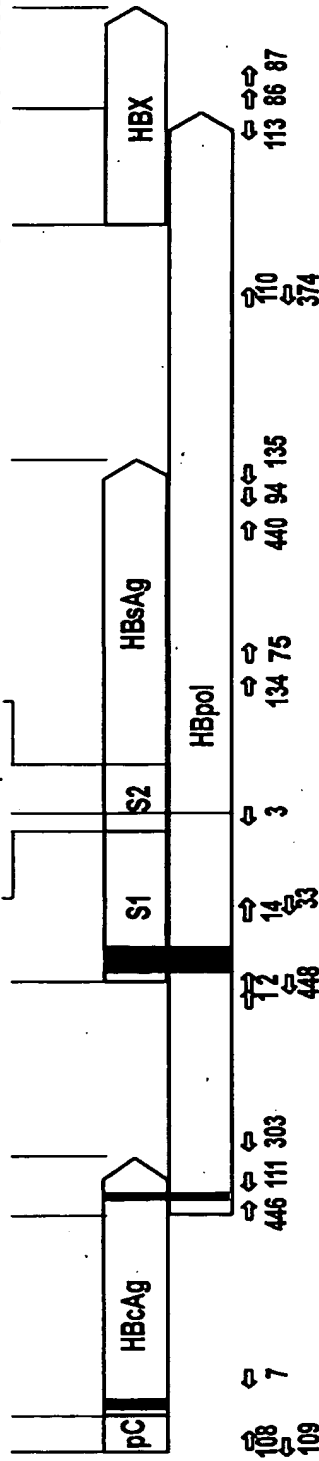
ATGGGGCTTT CTTGGACGGT CCCTCTCGAG TGNNGGGGAA
AGAACCTTTC CACCAGCAAT CCTCTAGGAT TCCTTCCC GA
TCACCAGTTG GACCCAGCAT TCAGAGCAAA TACCAACAAT
CCAGATTGGG ACTTCAATCC CAAAAGGAC CCTTGGCCAG
NAGCGAACAA GGTAGGAGTT GGAGCCTATG GACCCGGGTT
CACCCCTCCA CACGGAGGCC TTTTGGGGTG GAGCCCTCAG
TCTCAGGGCA CACTAACAAC TTTGCCAGCA GATCCGCCTC
CTGCCTCCAC CAATCGTCAG TCAGGGAGGC AGCCTACTCC
CATCTCTCCA CCACTAAGAG ACAGTCATCC TCAGGCCATG
CAGTGGAAC TACAGCATT CCACCAAGCT CTACAAAATC
CCAAAGTCAG GGGCCTGTAT TTTCTGTCTG GTGGCTCCAG
TTCAGGGATA GTGAACCCTG TTCCGACTAT TGCCTCTCAC
ATCTCGTCAA TCTTCTCCAG GATTGGGGAC CCTGCACCGA
ACATGGAGAA CATCACATCA GGATTCCTAG GACCCCTGCT
CGTGTTACAG GCGGGGTTTT TCTTGTTGAC AAGAATCCTC
ACAATACCGC AGAGTCTAGA CTCGTGGTGG ACTTCTCTCA
ATTTTCTAGG GGGAGTGCCC GTGTGTCCTG GCCTAAATTC
GCAGTCCCCA ACCTCCAATC ACTCACCAAT CTCCTGTCCT
CCAACCTGTC CTGGCTATCG CTGGATGTGT CTGCGGCGTT
TTATCATATT CCTCTTCATC CTGCTGCTAT GCCTCATCTT
CTTGTTGGTT CTTCTGGACT ATCAAGGTAT GTTGCCCGTT
TGTCTCTGA TTCCAGGATC CTCGACCACC AGTACGGGAC
CCTGCAAAAC CTGCACGACT CCTGCTCAAG GCAACTCTAT
GTATCCCTCA TGTGCTGTA CAAAACCTTC GGACGGAAAT
TGCACCTGTA TTCCCATCCC ATCGACTTGG GCTTTCGCAA
AATACCTATG GGAGTGGGCC TCAGTCCGTT TCTCTTGGCT
CAGTTTACTA GTGCCATTTG TTCAGTGGTT CGTAGGGCTT
TCCCCACTG TCTGGCTTTC AGCTATATGG ATGATGTGGT
ACTGGGGGCC AAGTCTGTAC AACATCTTGA GTCCCTTTAT
ACCGCTGTTA CCAATTTTCT TTTGTCTTAG GGTATACATT
TAA (SEQ ID NO 8)

Figure 3

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Nucleotide position.

A: 1814 1901	2307 2455	2854 3211	3221/1 155	832	1374	1620 1835
B: 1814 1901	2307 2449	2848 3205	3215/1 155	832	1374	1620 1835
C: 1814 1901	2307 2449	2848 3205	3215/1 155	832	1374	1620 1835
D: 1814 1901	2307 2449	2848 3172	3182/1 155	832	1374	1620 1835
E: 1814 1901	2307 2449	2848 3202	3212/1 155	832	1374	1620 1835
F: 1814 1901	2307 2449	2848 3205	3215/1 155	832	1374	1620 1835
G: 1814 1901	2343 2485	2884 3238	3248/1 155	832	1374	1620 1835



Total length in amino acid

	PreCore	HBcAg	PreS1	PreS2	HBsAg	HBpol	HBX
A:	29	185	119	55	226	845	154
B:	29	183	119	55	226	843	154
C:	29	183	119	55	226	843	154
D:	29	183	108	55	226	832	154
E:	29	183	118	55	226	842	154
F:	29	183	119	55	226	843	154
G:	29(1)	195	118	55	226	842	154

Figure 4

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PreS1

```

1      11      21      31      41      51      61      71      81      91      101      111
:      :      :      :      :      :      :      :      :      :      :      :
Gen A: MCGSSSKPRK GMGTNLSDVN PLGFPDPHQ L DPAGCANSNN PDWDENPKD HWPAAQVGV GAFGPRLTTP HGGILGWSQ AQGILITVST IPPPASTNRQ SGROPTPISP PLRDSHPQA
Gen B: -----Q-----K--D-----L--H--N--DS-K-----GF-----P--A-----L--K--L-----T-----
Gen C: -----Q-----R--TA-----N--N-----E--A-----GF-----V--PV A-----L-----NT-----
Gen D: -----LSWTV-LE W-K-I-TT-----R--TR-----H--N-----TE-K-----GF-----M-K-LPA D-----T-----
Gen E: -----APL-TT-R-----Q-----L-R--SS-----TN--S--M-K-----GY-GF-----V--LPA D-----L--K-QV-----T-----
Gen F: -----LSWTV-LE W-K--AS-----L-----R--T-----K--P--E-K-K-----Y-GF-----S--T--LPA D-----L-----
Gen G:

```

PreS2

```

1      11      21      31      41      51
:      :      :      :      :
Gen A: MCWNSTAFHQ ALQDPKVRGL YLPAGGSSSG TVNPAPNIAS HISSISARTG DPVTN
Gen B: -----T-----T--R--A--F-----VQ-T--S-----LST-----P-----
Gen C: -----T-----L--R-----F-----V--TT--P-----S-----AP-----
Gen D: -----T-----T--R-----F-----V--TT--P-----PS-I--AL-----
Gen E: -----T-----T--R-----F-----V--TT--L-----PS-I--AP-----
Gen F: -----H-----L--R--A--F-----Q-----T-----LT-----SK--G-AM-----
Gen G: -----N-----I--V--T-----I--V--T-----PS-I--AP-----

```

HBsAg

```

1      11      21      31      41      51      61      71      81      91      101      111
:      :      :      :      :      :      :      :      :      :      :      :
Gen A: MENITSGFLG PLLVIQAGFP LLTRILTIPO SLDSWNTSLN FLGGSPVCLG QNSQSPTS NH SPTSCPPICP GYRWMLRRP IIFLFIILLC LIFLVLDDY QGMPLVCPCLI PGSTTTSTGP
Gen B: -----A--L-----S--K--L-----ET-----QI-S-----C-----C-----S-----
Gen C: -----T-----K-----A-T-P-----TT-----T-----L--TS-----S-----
Gen D: -----S-----K-----A-----A-----L-R-P-----L-----L-----S-----
Gen E: -----L-----R--VC-----K-----V--P--L-----I--T-----L-----L-----S-----
Gen F: -----L-----R-----K-----L-----L-----L-----L-----L-----L-----S-----
Gen G: -----L-----R-----K-----L-----L-----L-----L-----L-----L-----S-----

```

```

121     131     141     151     161     171     181     191     201     211     221
:      :      :      :      :      :      :      :      :      :      :
Gen A: CKTCTTPAQG NSMPPSCCT KPTDGNCTCI PIPSSWAPAK YLWENASVRF SWLSLLVPFV QWFGVLSPTV WLSAIWMMWY WGRPLYSIVS PFIPLLPIPP CLWVYI (SEQ ID NO 15)
Gen B: -----T-----I-----T-----S-----S-----R--G-----G-----A-----A-----A-----Q-----C-----N-C-L-----N-N-L-----S-----
Gen C: -----I-----T-----S-----S-----R--G-----G-----A-----A-----A-----Q-----C-----N-C-L-----N-N-L-----S-----
Gen D: -----R--T-----T--Y-----S-----S-----R--G-----G-----A-----A-----A-----Q-----C-----N-C-L-----N-N-L-----S-----
Gen E: -----R--M-L-----T-----S-----S-----R--G-----G-----A-----A-----A-----Q-----C-----N-C-L-----N-N-L-----S-----
Gen F: -----AL-----T-----S-----S-----R--G-----G-----A-----A-----A-----Q-----C-----N-C-L-----N-N-L-----S-----
Gen G: -----Y-----S-----S-----R--G-----G-----A-----A-----A-----Q-----C-----N-C-L-----N-N-L-----S-----

```

Figure 6

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US5 GGAGCTACTGTGGAGTTGCTCTCGTTTTTTTGGCCTTCTGACTTTTTTCCGTCTGTTCTGATCTTCTCGACACCGCT
 US6 GGAGCTACTGTGGAGTTGCTCTCGTTTTTTGGCCTTCTGACTTTTTTCCGTCTGTTCTGATCTTCTCGACACCGCT
 US7 GGAGCTACTGTGGAGTTGCTCTCGTTTTTTGGCCTTCTGACTTTTTTCCGTCTGTTCTGATCTTCTCGACACCGCT
 US10 GGAGCTACTGTGGAGTTGCTCTCGTTTTTTGGCCTTCTGACTTTTTTCCGTCTGTTCTGATCTTCTCGACACCGCT

226

HBVXCPS TCAGCTCTGTATCGAGAAGCCTTAGAGTCTCTGAGCATTGCTCACCTCACCATACTGCATCAGGCAAGCCATT
 FR1 TCAGCTTTGTACCGGAATCCTTAGAGTCCCTCTGATCATTTGTTCCCTCACCATACAGCACTCAGGCAAGCAATC
 FR2 TCAGCTTTGTACCGGAATCCTTAGAGTCCCTCTGATCATTTGTTCCCTCACCATACAGCACTCAGGCAAGCAATC
 US1 TCAGCTTTGTACCGGAATCCTTAGAGTCCCTCTGATCATTTGTTCCCTCACCATACAGCACTCAGGCAAGCAATC
 US3 TCAGCTTTGTACCGGAATCCTTAGAGTCCCTCTGATCATTTGTTCCCTCACCATACAGCACTCAGGCAAGCAATC
 US5 TCAGCTTTGTACCGGAATCCTTAGAGTCCCTTTGATCATTTGTTCCCTCACCATACAGCACTCAGGCAAGCAATC
 US6 TCAGCTTTGTACCGGAATCCTTAGAGTCCCTCTGATCATTTGTTCCCTCACCATACAGCACTCAGGCAAGCAATC
 US7 TCAGCTTTGTACCGGAATCCTTAGAGTCCCTCTGATCATTTGTTCCCTCACCATACAGCACTCAGGCAAGCAATC
 US10 TCAGCTTTGTACCGGAATCCTTAGAGTCCCTCTGATCATTTGTTCCCTCACCATACAGCACTCAGGCAAGCAATC

301

HBVXCPS CTCTGCTGGGTGGAATTGATGACTCTAGCTACCTGGTGGGTAATAATTTGGAAGATCCAGCATCCAGGGATCTA
 FR1 CTGTGCTGGGTGAGTTGATGACTCTAGCTACCTGGTGGGTAATAATTTGGAAGATCCAGCATCCAGAGATTG
 FR2 CTGTGCTGGGTGAGTTGATGACTCTAGCTACCTGGTGGGTAATAATTTGGAAGATCCAGCATCCAGAGATTG
 US1 CTGTGCTGGGTGAGTTGATGACTCTAGCTACCTGGTGGGTAATAATTTGGAAGATCCAGCATCCAGAGATTG
 US3 CTGTGCTGGGTGAGTTGATGACTCTAGCTACCTGGTGGGTAATAATTTGGAAGATCCAGCATCCAGAGATTG
 US5 CTGTGCTGGGTGAGTTGATRACTCTAGCTACCTGGTGGGTAATAATTTGGAAGATCCAGCATCCAGAGATTG
 US6 CTGTGCTGGGTGAGTTGATGACTCTAGCCACCTGGTGGGTAATAATTTGGAAGATCCAGCATCCAGAGATTG
 US7 CTGTGCTGGGTGAGTTGATGACTCTAGCTACCTGGTGGGTAATAATTTGGAAGATCCAGCATCCAGAGATTG
 US10 CTGTGCTGGGTGAGTTGATGACTCTAGCTACCTGGTGGGTAATAATTTGGAAGATCCAGCATCCAGAGATTG

Figure 7 - cont'd 1

376
 HBVXCPS GTAGTCAATTATGTTAATACTAACAATGGGTTTAAAGATCAGGCAACTATTGTGGTTTTCATATATCTTGCCCTTACT
 FR1 GTGGTCAATTATGTTAATACTAATAATGGGTTTAAATAATCAGGCAACTATTGTGGTTTTCACATTTCCCTGTCTTACT
 FR2 GTGGTCAATTATGTTAATACTAATAATGGGTTTAAATAATCAGGCAACTATTGTGGTTTTCACATTTCCCTGTCTTACT
 US1 GTGGTCAATTATGTTAATACTAATAATGGGTTTAAATAATCAGGCAACTATTGTGGTTTTCACATTTCCCTGTCTTACT
 US3 GTGGTCAATTATGTTAATACTAATAATGGGTTTAAATAATCAGGCAACTATTGTGGTTTTCACATTTCCCTGTCTTACT
 US5 GTGGTCRAATTATGTTAATACTAATAATGGGTTTAAATAATCAGGCARCGATTGTGGTTTTCACATCTCCTGTCTTACT
 US6 GTGGTCAATTATGTTAATACTAATAATGGGTTTAAATAATCAGGCAACTATTGTGGTTTTCACATTTCCCTGTCTTACT
 US7 GTGGTCAATTATGTTAATACTAATAATGGGTTTAAATAATCAGGCAACTATTGTGGTTTTCACATTTCCCTGTCTTACT
 US10 GTGGTCAATTATGTTAATACTAATAATGGGTTTAAATAATCAGGCAACTATTGTGGTTTTCACATTTCCCTGTCTTACT

451
 HBVXCPS TTTGGAAGAGAGACTGTGCTTGAATATTGGTCTCTTTCCGGAGTGTGGATTCCGCACTCCTCCAGCCCTATAGACCA
 FR1 TTTGGGAGAGAAAACCGTTCTTGAGTATTGGTGTCTTTTGGAGTGTGGATTTCGCACTCCTCCTGTCTTATAGACCA
 FR2 TTTGGGAGAGAAAACCGTTCTTGAGTATTGGTGTCTTTTGGAGTGTGGATTTCGCACTCCTCCTGTCTTATAGACCA
 US1 TTTGGGAGAGAAAACCGTTCTTGAGTATTGGTGTCTTTTGGAGTGTGGATTTCGCACTCCTCCTGTCTTATAGACCA
 US3 TTTGGGAGAGAAAACCGTTCTTGAGTATTGGTGTCTTTTGGAGTGTGGATTTCGCACTCCTCCTGTCTTATAGACCA
 US5 WTWGGGASAGAAACCGTTCTTGAGTATTGGTGTCTWTKKGGAGTGTGSAATTCGCACTCCTCMTGCTTATAGACCA
 US6 TTTGGGAGAGAAAACCGTTCTTGAGTATTGGTGTCTTTTGGAGTGTGGATTTCGCACTCCTCCTGTCTTATAGACCA
 US7 TTTGGGAGAGAAAACCGTTCTTGAGTATTGGTGTCTTTTGGAGTGTGGATTTCGCACTCCTCCTGTCTTATAGACCA
 US10 TTTGGGAGAGAAAACCGTTCTTGAGTATTGGTGTCTTTTGGAGTGTGGATTTCGCACTCCTCCTGTCTTATAGACCA

526
 HBVXCPS CCAAAATGCCCTATCTTATCAACACTTCCGGAAACTACTGTTGTTAGACGACGGGACCGAGGCAGGTCCCCCTAGA
 FR1 CCAAAATGCCCTATCTTATCAACACTTCCGGAGACTACTGTTGTTAGACGAAGNNNNNNNAGGCAGGTCCCCCTCGA
 FR2 CCAAAATGCCCTATCTTATCAACACTTCCGGAGACTACTGTTGTTAGACGAAGNNNNNNNAGGCAGGTCCCCCTCGA
 US1 CCAAAATGCCCTATCTTATCAACACTTCCGGAGACTACTGTTGTTAGACGAAGNNNNNNNAGGCAGGTCCCCCTCGA

Figure 7 - cont'd 2

22/34

US3 CCAATGCCCCCTATCCTATCAACACTTCGGAGACTACTGTTGTTAGACGAAGNNNNNNAGGCAGGTCCCCTCGA
 US5 CCAATGCCCCCWATCCYATCAACACTTCGGAGRCTRCTGTTGTTAGRCGAAGNNNNNNAGGCAGGTCCCCTCGA
 US6 CCAATGCCCCCTATCCTATCAACACTTCGGAGACTACTGTTGTTAGACGAAGNNNNNNAGGCAGGTCCCCTCGA
 US7 CCAATGCCCCCTATCCTATCAACACTTCGGAGACTACTGTTGTTAGACGAAGNNNNNNAGGCAGGTCCCCTCGA
 US10 CCAATGCCCCCTATCCTATCAACACTTCGGAGACTACTGTTGTTAGACGAAGNNNNNNAGGCAGGTCCCCTCGA

601
 HBVXCPS AGAAGAACTCCCTCGCTCGCAGACGCAGATCTCAATCGCCGCGTCGCAGAAGATCTCAATCTCGGAATCTCAA
 FR1 AGAAGAACTCCCTCGCTCGCAGACGAAGATCTCAATCGCCGCGTCGCAGAAGATCTGCATCTCCAGCTTCCCCAA
 FR2 AGAAGAACTCCCTCGCTCGCAGACGAAGATCTCAATCGCCGCGTCGCAGAAGAT (SEQ ID NO 45)
 US1 AGAAGAACTCCCTCGCTCGCAGACGAAGATCTCAATCGCCGCGTCGCAGAAGAT (SEQ ID NO 46)
 US3 AGAAGAACTCCCTCGCTCGCAGACGAAGATCTCAATCGCCGCGTCGCAGAAGAT (SEQ ID NO 47)
 US5 AGAAGAACTCCCTCGCTCGCAGACGAAGATCTCAATCGCCGCGTCGCAGAAGAT (SEQ ID NO 48)
 US6 AGAAGAACTCCCTCGCTCGCAGACGAAGATCTCAATCGCCGCGTCGCAGAAGATATGCATCTC
 US7 AGAAGAACTCCCTCGCTCGCAGACGAAGATCTCAATCGCCGCGTCGCAGAAGAT (SEQ ID NO 50)
 US10 AGAAGAACTCCCTCGCTCGCAGACGAAGATCTCAATCGCCGCGTCGCAGAAGAT (SEQ ID NO 51)

676
 HBVXCPS TGTTAG (SEQ ID NO 43)
 FR1 TGTTAG (SEQ ID NO 44)
 US6 (SEQ ID NO 49)

Figure 7 - cont'd 3

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1
 HBVXCPS ATGGGAGGTTGGTCATCAAAACCTCGCAAGGCATGGGACGAATCTTTCTGTTCCTCCAAACCTCTGGGATTCCTTT
 FR1 -----GCT--CT-GGA-GGTC-----T-G-GNNNTG--A-A--C-----C-CCAG--T-----A-----C--
 FR2 T--CT-GGA-GGTC-----T-G-GNNNTG--A-A--C-----CACCAG--T-----A-----C--
 US1 GTC-----T-G-GNNNTG--A-A--C-----CACCAG--T-----A-----C--
 US3 CGTC-----T-G-GNNNTG--A-A--C-----CACCAG--T-----A-----C--
 US6 C-----T-G-GNNNTG--A-G--C-----CACCAG--T-----A-----C--
 US7 C-----T-G-GNNNTG--A-A--C-----CACCAG--T-----A-----C--
 US9 G-GNNNTG--A-A--C-----CACCAG--T-----A-----C--
 US10 T-GGCGG--CTCTG-GNNNTG--A-A--C-----CACCAG--T-----A-----C--

76
 HBVXCPS CCCGATCATCAGTTGGACCCCTGCATTCGGAGCCAACTCAAACAATCCAGATTGGGACTTCAACCCCATCAAGGAC
 FR1 -----C-----A-----A--A--TA-C-----T-----T-----AA-----
 FR2 -----C-----A-----A--A--TA-C-----T-----T-----AA-----
 US1 -----C-----A-----A--A--TA-C-----T-----T-----AA-----
 US3 -----C-----A-----A--A--TA-C-----T-----T-----AA-----
 US6 -----C--A--T-----A-----A--TA-C-----T-----T-----AA-----
 US7 -----C-----A-----A--A--TA-C-----T-----T-----AA-----
 US9 -----C-----A-----A--A--TA-C-----T-----T-----AA-----
 US10 -----C-----A-----A--A--TA-C-----T-----T-----AA-----

151
 HBVXCPS CACTGGCCAGCAGCCAGGTAGGAGTGGAGCATTCGGGCCAAGGCTCACCCCTCCACACGGCGGTATTTTG
 FR1 -CT-----AG-----A-----T-----C-AT--A--CG--T-----A--CC-----
 FR2 -CT-----AG-----A-----T-----C-AT--A--CG--T-----A--CC-----

Figure 8

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US1 -CT-----AG-----A-----T-----C-AT--A--CG--T-----A--CC-----
 US3 -CT-----AG-----A-----T-----C-AT--A--CG--T-----A--CC-----
 US6 -CT-----AG-----A-----T-----C-AT--A--CG--T-----A--CC-----
 US7 -CT-----AG-----A-----T-----C-AT--A--CG--T-----A--CC-----
 US9 -CT-----AG-----A-----T-----C-AT--A--CG--T-----A--CC-----
 US10 -CT-----AG-----A-----T-----C-AT--A--CG--T-----A--CC-----

226

HBVXCPS GGGTGGAGCCCTCAGGCTCAGGGCATATTGACCACAGTGTCAACAATTCTCTCCTGCCCTCCACCAATCGGCAG
 FR1 -----T-----C-C-A--A--TT--C--G--GA--G-----T-----
 FR2 -----T-----C-C-A--A--TT--C--G--GA--G-----T-----
 US1 -----T-----C-C-A--A--TT--C--G--GA--G-----T-----
 US3 -----T-----C-C-A--A--TT--C--G--GA--G-----T-----
 US6 -----T-----C-C-A--A--TT--C--G--GA--G-----T-----
 US7 -----T-----C-C-A--A--TT--C--G--GA--G-----T-----
 US9 -----T-----C-C-A--A--TT--C--G--GA--G-----T-----
 US10 -----T-----C-C-A--A--TT--C--G--GA--G-----T-----

301

HBVXCPS TCAGGAAGGCAGCCTACTCCCATCTCTCCACCTCTAAGAGACAGTCACTCTCAGGCCATGCAGTGGAATTCCTACT
 FR1 -----G-----A-----A-----K-----Y-----C--T--A
 FR2 -----G-----A-----A-----K-----Y-----C--T--A
 US1 -----G-----A-----A-----A-----C-----C--T--A
 US3 -----G-----A-----A-----A-----C-----C--T--A
 US6 -----G-----A-----A-----A-----C-----C--T--A

Figure 8 - cont'd 1

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US7 G-----A-----C-T-A
 US9 G-----A-----C-T-A
 US10 G-----A-----C-T-A

376

HBVXCPS GCCTTCCACCAAGCTCTGCAGGATCCCAAAGTCAGGGTCTGTATCTTCTGCTGGTCCAGTTCAGGAACA
 FR1 A-----A-AA-----C-----T-----G-T-
 FR2 A-----A-AA-----C-----T-----G-T-
 US1 A-----A-AA-----C-----T-----G-T-
 US3 A-----A-AA-----C-----T-----A-G-T-
 US6 A-----A-AA-----C-----T-----G-T-
 US7 A-----A-AA-----C-----T-----G-T-
 US9 A-----A-AA-----C-----T-----G-T-
 US10 A-----A-AA-----GCCNNNNNNNNNN-----G-T-

451

HBVXCPS GTAAACCCTGCTCCGAATATTGCCCTCTCACATCTCGTCAATCTCCGCGAGGACTGGGGACCCCTGTGACGAACATG
 FR1 G-----T-----C-----T-T-C-----CAC-
 FR2 G-----T-----C-----T-T-C-----CAC-
 US1 G-----T-----C-----T-T-C-----CAC-
 US3 G-----T-----C-----T-T-C-----CAC-
 US6 G-----T-----C-----T-T-C-----CAC-
 US7 G-----T-----C-----T-T-C-----CAC-
 US9 G-----T-----C-----T-T-C-----CAC-
 US10 G-----T-----C-----T-T-C-----CAC-

Figure 8 - cont'd 2

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526

HBVXCPS GAGAACATCACATCAGGATTCCCTAGGACCCCTGCTCGTGTACAGGCGGGGTTTCTTGTGACAAGAATCCTC
 FR1 -----
 FR2 -----
 US1 -----
 US3 -----T-----
 US6 -----
 US7 -----
 US9 -----
 US10 -----

601

HBVXCPS ACAATACCGCAGAGTCTAGACTCGTGGTGGACTTCTCTCAATTTCTAGGGGGTCAACCGTGTCTTGGCCAA
 FR1 -----AGTG-----C-----T-----
 FR2 -----AGTG-----C-----T-----
 US1 -----AAGTG-----C-----TT-----
 US3 -----AGTG-----C-----T-----
 US6 -----AGTG-----C-----T-----
 US7 -----AGTG-----C-----T-----
 US9 -----AGTG-----C-----T-----
 US10 -----AGTG-----C-----T-----

676

HBVXCPS AATTGCGAGTCCCCAACCTCCAATCACTCACCACCTCCTGTCTCCAATTTGTCTCGTTATCGCTGGATGTGT
 FR1 -----T-----C-----C-----
 FR2 -----T-----C-----C-----

Figure 8 - cont'd 3

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US1 -----T-----C-----C-----
US3 -----T-----C-----C-----
US6 -----T-----C-----C-----
US7 -----T-----C-----C-----
US9 -----T-----C-----C-----
US10 -----T-----C-----C-----

751
HBVXCPS CTGCGGCGTTTATCATATTCCTCTTCATCCCTGCTGCTATGCCCTCATCTTCTTGGTTCTTCTGGATTATCAA
FR1 -----C-----C-----
FR2 -----C-----C-----
US1 -----C-----C-----
US3 -----C-----C-----
US6 -----C-----C-----
US7 -----C-----C-----
US9 -----C-----C-----
US10 -----C-----C-----

826
HBVXCPS GGTATGTTGCCCGTTTGTCCCTCTAATCCAGGATCAACAACCAAGTACGGGACCATGCAAAACCTGCACGACT
FR1 -----G-----CT-G-C-----C-----
FR2 -----G-----CT-G-C-----C-----
US1 -----G-----CT-G-C-----C-----
US3 -----G-----CT-G-C-----C-----
US6 -----G-----CT-G-C-----C-----

Figure 8 - cont'd 4

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US7 -----G-----CT-G-C-----C-----
US9 -----G-----CT-G-C-----C-----
US10 -----G-----CT-G-C-----C-----

901

HBVXCPS CCTGCTCAGGCAACTCTATGTTCCCTCATGTTGCTGTACAAAACCTACGGATGGAAATTGCACCTGTATTCCC
FR1 -----A-----T-----C-----
FR2 -----A-----T-----C-----
US1 -----A-----T-----C-----
US3 -----A-----T-----C-----
US6 -----A-----T-----C-----
US7 -----A-----T-----C-----
US9 -----A-----T-----C-----
US10 -----A-----T-----C-----

976

HBVXCPS ATCCCATCGTCCCTGGGCTTTCGCAAAATACCTATGGGAGTGGGCCCTCAGTCCGTTTCTCTTGGCTCAGTTACTA
FR1 -----A-T-----
FR2 -----A-T-----
US1 -----A-T-----
US3 -----A-T-----
US6 -----A-T-----
US7 -----A-T-----
US9 -----A-T-----
US10 -----A-T-----

Figure 8 - cont'd 5

1051

HBVXCPS GTGCCATTGTTTCAGTGGTTCGTTAGGGCTTCCCCCACTGTTTGGCTTTCAGCTATATGGATGATGGTATTGG
 FR1 -----C-----
 FR2 -----C-----
 US1 -----C----- (SEQ ID NO 55)
 US3 -----C----- (SEQ ID NO 56)
 US6 -----C-----
 US7 -----M----- (SEQ ID NO 58)
 US9 -----C-----
 US10 -----G-T-CGAGG-CTT-C (SEQ ID NO 60)

1126

HBVXCPS GGGCCAA (SEQ ID NO 52)
 FR1 ----- (SEQ ID NO 53)
 FR2 ----- (SEQ ID NO 54)
 US6 -- (SEQ ID NO 57)
 US9 (SEQ ID NO 59)

Figure 8 - cont'd 6

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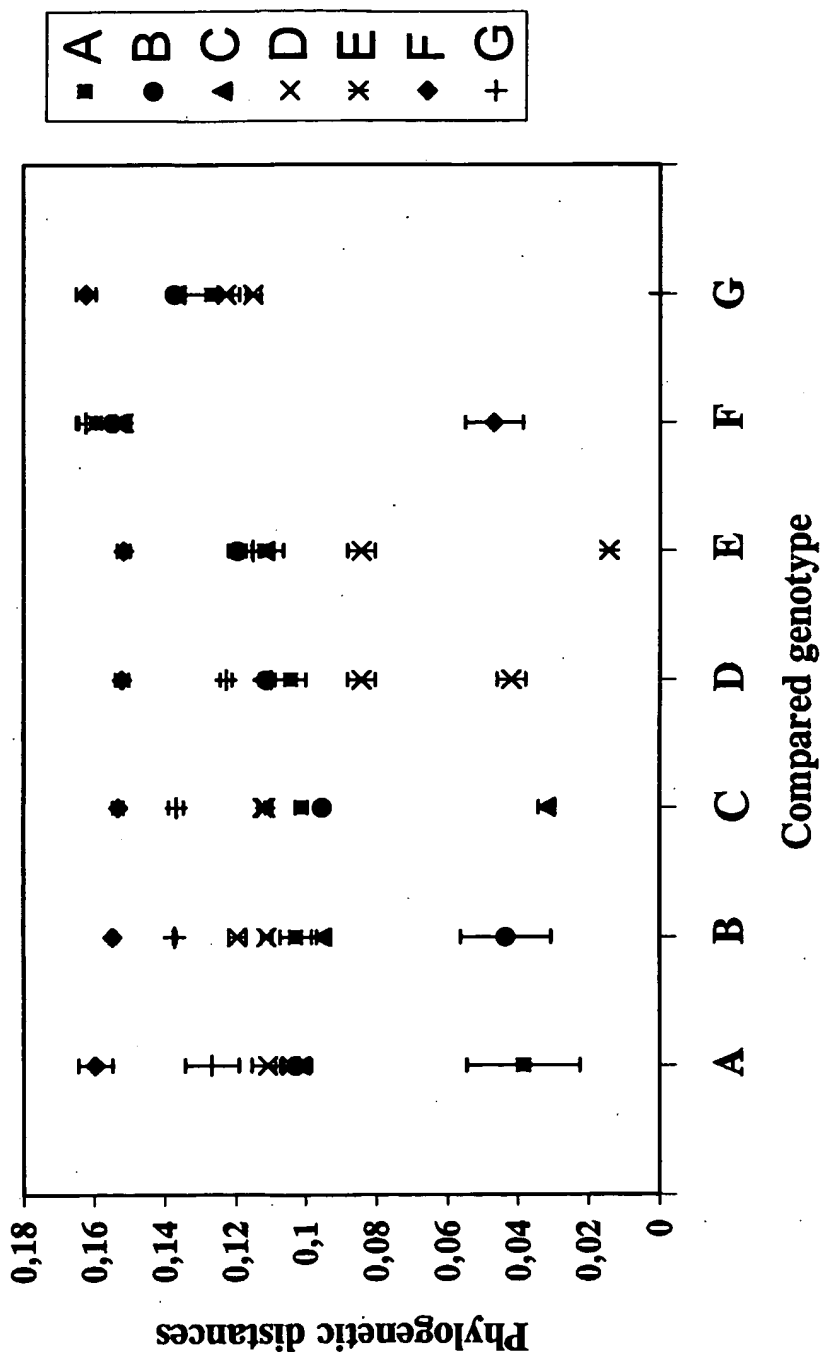


Figure 9

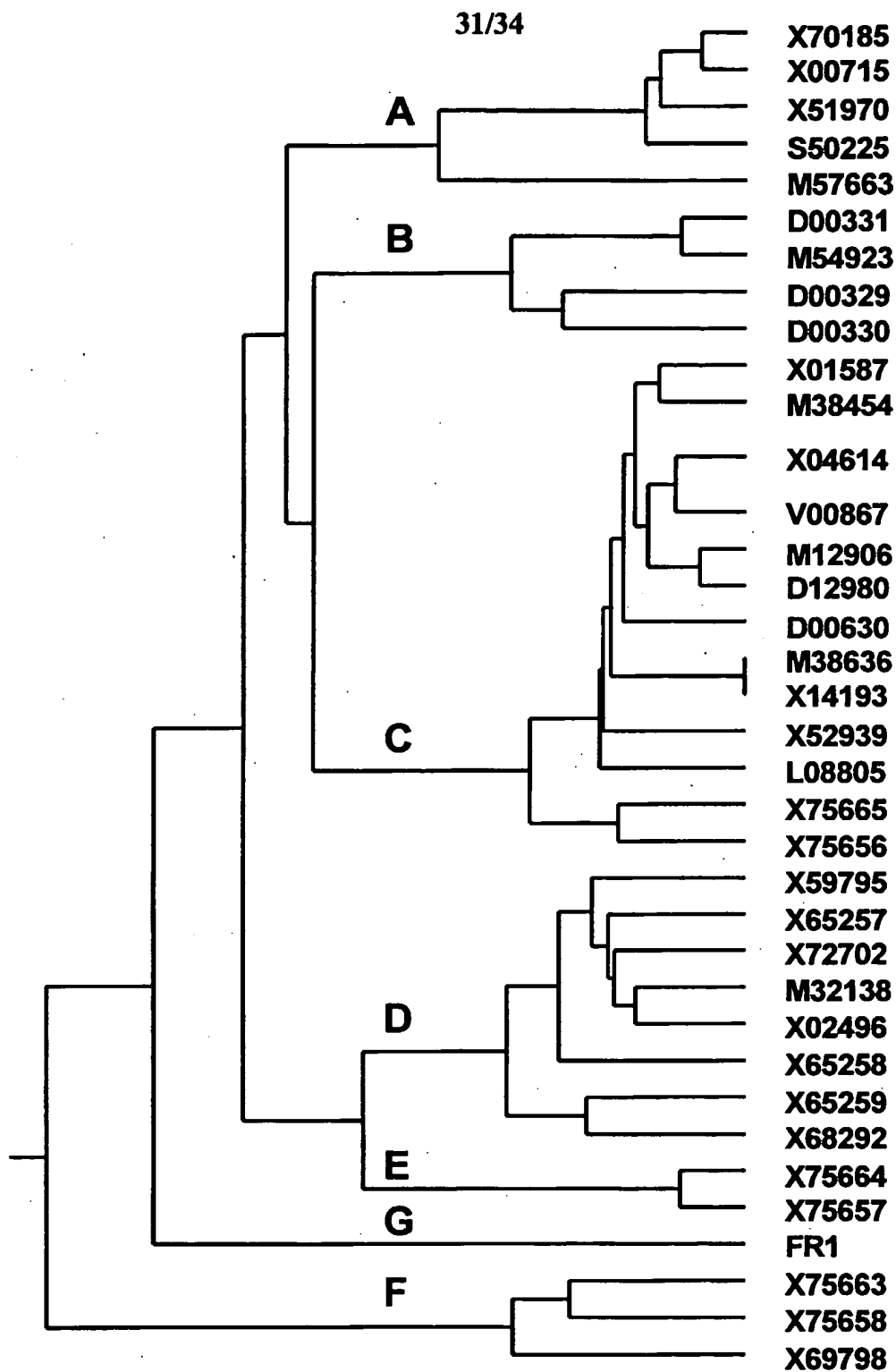


Figure 10a

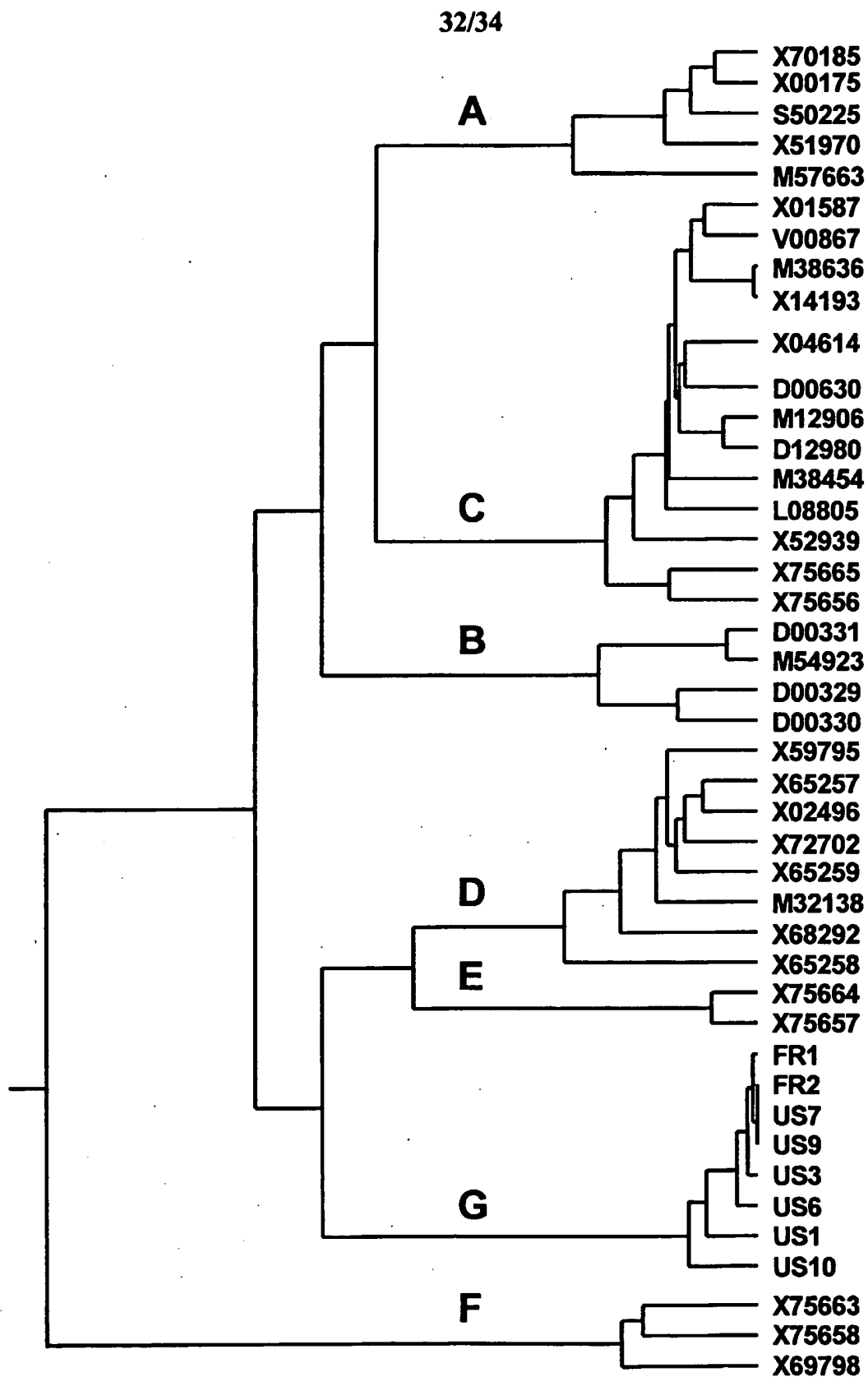


Figure 10 b

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Genotype

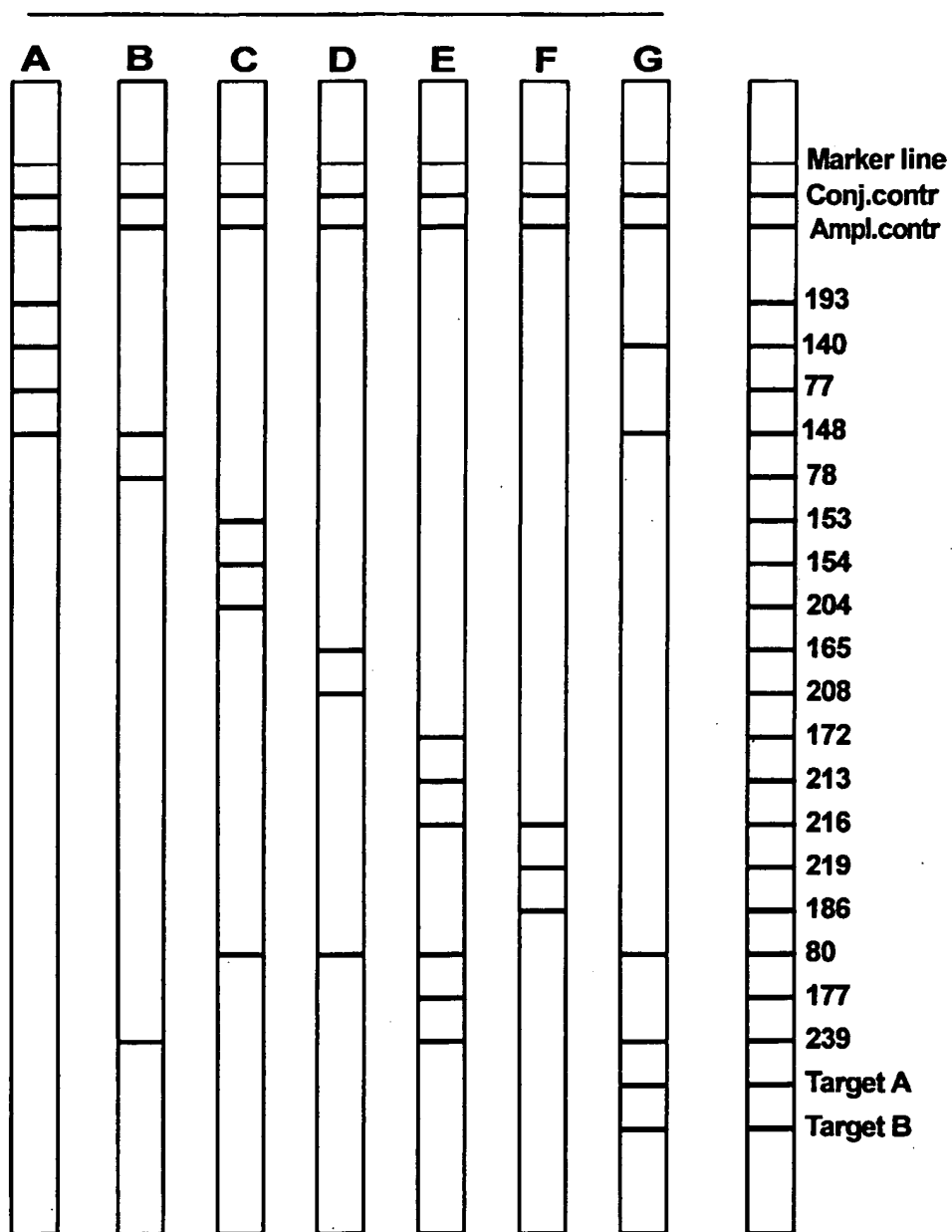


Figure 11

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ATGTAAC TTT TTCACCTCTG CCTAATCATC TCTTGTTTCAT
GTCCTACTGT TCAAGCCTCC AAGCTGTGCC TTGGGTGGCT
TTAGGGCATG GATAGAACAA CTTTGCCATA TGGCCTTTTT
GGCTTAGACA TTGACCCTTA TAAAGAATTT GGAGCTACTG
TGGAGTTACT CTCGTTTTTG CCTTCTGACT TTTTCCCGTC
TGTTTCGTGAT CTTCTCGACA CCGCTTCAGC TTTGTACCGG
GAATCCTTAG AGTCCTCTGA TCATTGTTTCG CCTCACCATA
CAGCACTCAG GCAAGCAATC CTGTGCTGGG TGGAGTTGAT
GACTCTAGCT ACCTGGGTGG GTAATAATTT GGAAGATCCA
GCATCCAGAG ATTTGGTGGT CAATTATGTT AATACTAATA
TGGGTTTAAA AATCAGGCAA CTATTGTGGT TTCACATTTT
CTGTCTTACT TTTGGAAGAG AAACCGTTCT TGAGTATTTG
GTGTCTTTTG GAGTGTGGAT TCGCACTCCT CCTGCTTATA
GACCACCAA TGCCCCTATC CTATCAACAC TTCCGGAGAC
TACTGTTGTT AGACGAAGAG GCAGGTCCCC TAGAAGAAGA
ACTCCCTCGC CTCGCAGACG CAGATCTCCA TCGCCGCGTC
GCAGAAGATC TCAATCTCGG GAATCTCAAT GTTAG
(SEQ ID NO 169)

Figure 12